

AD _____

AWARD NUMBER: W81XWH-07-1-0011

TITLE: Development of a Minimal-Bulk Oxygen Delivery Product to Enhance Survival During Hemorrhagic Shock/Studies Regarding the Use of Perfluorocarbon-Derived Intravascular Microbubbles from Oxygen Transport

PRINCIPAL INVESTIGATOR: Claes Lundgren M.D., Ph.D.
Ingvard Tyssebotn M.D., Ph.D.

CONTRACTING ORGANIZATION: Research Foundation, State of New York,
Buffalo, NY 14226

REPORT DATE: July 2009

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE 1 July 2009	2. REPORT TYPE Final	3. DATES COVERED 16 Jan 2007 – 15 Dec 2008		
4. TITLE AND SUBTITLE Development of a Minimal-Bulk Oxygen Delivery Product to Enhance Survival During Hemorrhagic Shock/Studies Regarding the Use of Perfluorocarbon-Derived Intravascular Microbubbles from Oxygen Transport		5a. CONTRACT NUMBER		
		5b. GRANT NUMBER W81XWH-07-1-0011		
		5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Claes Lundgren M.D, Ph.D.; Ingvald Tyssebotn M.D., Ph.D. E-Mail: ityssebo@buffalo.edu		5d. PROJECT NUMBER		
		5e. TASK NUMBER		
		5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Research Foundation, State of New York Buffalo, NY 14226		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)		
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				
13. SUPPLEMENTARY NOTES				
14. ABSTRACT <p>Goal 1: Production of microbubble forming dodecafluoropentane emulsion (DDFPe); NuvOx Pharma LLC, Tucson, AZ was able to make high-quality emulsions February 2008, which have been successfully used in resuscitation of rats (sever hemodilution) and pigs (potentially lethal hemorrhagic shock) over the last 16 months. Goal 2: A shock model combining hemorrhage (~32 mL/kg total blood loss) and a fractured femur bone was developed in pentobarbital anesthetized pigs weighing approximately 25 kg. In 19 pigs not given any treatment, ~30% of the pigs survived for six hours. Goal 3: Comparison of Hextend (plasma expander) and DDFPe (blood substitute) for low volume resuscitation in the model developed during Goal 2. Eight out of ten pigs survived if Hextend was given 45 minutes after bleeding, suggesting a survival of ~80%. All six pigs given 0.6 mL/kg DDFPe (NuvOx Pharma LLC) survived with good physiological values for more than six hours. This was interpreted as 100% of the DDFPe treated pigs survived for the required time. Repeatedly observed in Hextend treated pigs was development of severe pulmonary distress with foam in the lungs, dyspnea, and gradually rising content of CO₂ and falling O₂ tension in arterial blood. Goal 4: Semi-conscious pigs were bled ~31.5 mL/kg over 26 min and were allowed to develop shock over the next ~35 min. Two randomly selected groups (n=6 each) were resuscitated with either 7 mL/kg Hextend or 0.6 mL/kg DDFPe over 30 min. One pig died before any treatment was given. All treated pigs survived the experiments for 11 to 14 days, then euthanized. One of the Hextend treated pigs suffered severe sequel from the shock with no weight gain and was required euthanization on the 11th day. All DDFPe treated pigs were in good condition, increasing their weight at a normal rate. Goal 5: Pigs (from model described in Goal 2) in hemorrhagic shock were successfully resuscitated with Hextend or DDFPe given over 15 min. Conclusion: The experiments performed show that DDFPe emulsion is a better resuscitation fluid than Hextend.</p>				
15. SUBJECT TERMS Hemorrhagic shock, broken bone, dodecafluoropentane emulsion, Hextend, low volume resuscitation, semiconscious pigs				
16. SECURITY CLASSIFICATION OF: a. REPORT U		17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 49	19a. NAME OF RESPONSIBLE PERSON USAMRMC
b. ABSTRACT U				19b. TELEPHONE NUMBER (include area code)
c. THIS PAGE U				

TABLE OF CONTENTS

	<u>Page number</u>
INTRODUCTION	3
BODY OF WORK	3
THE AIMS OF THE STUDY	3
Aim 1 - Development of new DDFP emulsion (<i>Goal 1</i>)	4
Aim 2 - DDFP emulsion and Hextend as resuscitation fluids	4
METHODS	5
TESTING OF DDFP EMULSIONS ON RATS	5
MODEL I: HEMORRHAGE IN ANAESTHETIZED PIGS WITH BROKEN FEMUR AND LAPAROTOMY	5
MODEL II: HEMORRHAGE IN SEDATED PIGS - COMPARISON OF DDFPe TREATMENT AND HEXTEND TREATMENT	5
The timeline for sedated pig experiments	5
The training/habituation program	6
Surgical preparation - Day 1	6
Hemorrhagic shock experimental procedure - Day 2 or 3	6
Physiological measurements and euthanasia - Day 11 or 14	7
MODEL III - RAPID INFUSIONS OF DDFPe AND HEXTEND	8
STATISTICS AND CALCULATIONS	8
RESULTS	8
MODEL II - SEDATED PIGS GIVEN DDFPe OR HEXTEND TREATMENTS	8
The training/ habituation	8
Day 1 - Physiological measurements during anesthesia	9
Day 2 - Hemorrhagic shock development and treatments during light sedation	9
<u>HEXTEND TREATED PIGS</u>	9
<u>DDFPe TREATED PIGS</u>	9
<u>COMPARISON BETWEEN THE TWO GROUPS OF ANIMALS</u>	9
Day 14 – Physiological measurements, euthanasia, and necropsy	10
MODEL III - INCREASED INFUSION RATE DURING DDFPe OR HEXTEND TREATMENT	10
DISCUSSION	11
KEY RESEARCH ACCOMPLISHMENTS	12
REPORTABLE OUTCOME	12
CONCLUSIONS	12
SO WHAT	13
PROBLEMS MET AND SOLVED DURING THE PROJECT	13

REFERENCES	14
APPENDICES	15
1 - FIGURES WITH LEGENDS	15
2 – PROOF OF LICENSING	34
3 - CURRICULUM VITAE (for Principal Investigator)	35

INTRODUCTION

The proposal for the project "Development of a Minimal-Bulk Oxygen Delivery Product to Enhance Survival During Hemorrhagic Shock", contract # W81XWH-07-1-0011, was submitted by Dr. Claes Lundgren MD, PhD (Director of CRESE and Distinguished Professor at Department of Physiology and Biophysics, SUNY at Buffalo, NY) and Dr. Evan Unger MD, PhD (CO of ImaRx Pharmaceuticals, Inc., Tucson, AZ) as Principal Investigators (PI).

Dr. Lundgren withdrew as PI from the project in the fall of 2007, due to retirement, and the project was continued by Dr. Ingvald Tyssebotn MD, PhD as PI. Dr. Evan Unger resigned from ImaRx Pharmaceuticals, Inc. shortly after signing the contract with the US Army. A new agreement of production of DDFP emulsion was negotiated between the University of Buffalo and NuvOx Pharma, LLC and signed in January 2008 (see Appendices).

The project was brought forward with the title: Development of a Minimal-Bulk Oxygen Delivery Product to Enhance Survival during Hemorrhagic Shock/Studies regarding the use of Perfluorocarbon derived intravascular microbubbles for oxygen transport.

The US forces have been searching for the optimal treatment of hemorrhagic shock for decades. The standard treatment for a wounded soldier in the field has recently been to stop the bleeding and give 500 mL of Hextend (6 % hetastarch in lactated electrolyte injection; Hespira Inc., Lake Forest, IL) as soon as a paramedic arrives. Assuming a standard person weighs 70 kg, the dose of the plasma expander, Hextend, will be 7 mL/kg. To imitate this scenario, we have developed a model on anesthetized pigs (**Model I**) with a broken bone and hemorrhagic shock, comparing treatment with intravascular oxygen-carrying micro bubbles of dodecafluoropentane emulsion (DDFPe), 0.6 mL/kg, and treatment with Hextend, 7 mL/kg. A survival model (**Model II**) was developed where pigs had a venous and an arterial catheter implanted during a brief Isoflurane anesthesia 2-3 days before the bleeding, and the hemorrhage performed while the pigs were tranquilized and semi-conscious. The pigs were treated by either Hextend or DDFPe, in the same doses as in **Model I**, and the pigs kept alive for the next 11 to 14 days. In **Model III** the treatment volumes were similar, but the infusion rate was doubled (15 min, compared to 30 min).

BODY OF WORK

THE AIMS OF THE STUDY

Aim 1 (Goal 1): To optimize, evaluate and manufacture, on a laboratory scale, a novel oxygen transporting erythrocyte replacement intravenous preparation consisting of a 2% DDFPe stabilized with PEG Telomer B, Pluronic P123 and sucrose.

Aim 2 (Goal 2 to 5): To demonstrate the unique properties of the preparation (produced under Goal 1) as low volume resuscitation fluid for treatment of hemorrhagic shock in field-realistic animal models.

Aim 1 - Development of new DDFP emulsion (*Goal 1*)

The production of 2% DDFPe terminated in September 2007 and the manufacturer ImaRx Pharmaceuticals, Inc. (Tucson, AZ) withdrew from the project in the fall of 2007. Another small company, NuvOx Pharma, LLC (Tucson, AZ) bought the production rights and negotiated a contract with State University of New York at Buffalo by UB Office of Science, Technology Transfer and Economic Outreach early 2008. The company was able to start production of the new formula in February 2008, a replica of the previously produced EchoGen, Sonus Pharmaceuticals, Inc., Bothell, WA. This emulsion is now 15 months old and the particles have been stable and so far have not shown signs to deteriorate or to grow in size. The emulsion has been stored at 25°C with 60% relative humidity and at 40°C with 75% relative humidity since production, but still not close to the planned 3 years (The EchoGen particles were mainly stable for 11 years when stored in room temperature after the production in June 1997). The new formula has been used intensively over these months in pigs and rats, and has been found to be as good as the EchoGen produced by Abbott in 1997. The new emulsion passed our rigorous tests in a hemodiluted rat model (described by Lundgren, Bergoe and Tyssebotn, 2006) with survival of 5 rats for 2 hours at hemoglobin of less than 2.0 g/100 mL.

The screening of the DDFPe formulation produced by ImaRx Pharmaceuticals, Inc., was reported on March 2008.

All work and results from here on are discussed in relation to the microbubble-producing DDFPe (NVP-108) manufactured by NuvOx Pharma, LLC, Tucson, AZ.

Aim 2 - DDFP emulsion and Hextend as resuscitation fluids

To show the unique properties of the DDFPe that creates microbubbles when heated above 29°C in a warm-blooded body, the bubbles were used for treatment of hemorrhagic shock in several animal models. During the first passage through the lungs, the bubbles (diameter: 2-3 µm) take up oxygen from the alveoli similar to red blood cells. The bubbles are transported with the blood to the tissues where oxygen is unloaded and carbon dioxide absorbed for transportation back to the lungs (Burkard and Van Liew, 1994). The size of the microbubbles is determined by the size of the emulsion particles (~200 nm in diameter, NuvOx Pharma, LLC).

The studies in aim 2 were divided into four different goals using three different animal models all with pathogen free Yorkshire pigs.

Goal 2: Develop an animal model that after hemorrhagic shock combined with a broken femur, soft tissue injuries and laparotomy with no resuscitation will give a survival rate of ~30% (**Model I**)

Goal 3: Comparison of DDFPe treatment and Hextend treatment in **Model I** (reported March 2008).

Goal 4: Study the long term consequences of hemorrhagic shock in slightly sedated pigs (surviving 14 days) (**Model II**). Comparison of resuscitation with DDFPe treatment and Hextend treatment in **Model II**.

Goal 5: Evaluation of increased infusion rates on the outcome of DDFPe and Hextend treatments using the experimental setup as in Model I (**Model III**)

METHODS

TESTING OF DDFP EMULSIONS ON RATS

In this project, the two NuvOx Pharma, LLC DDFP emulsions produced mid-February, 2008, were tested on eight Wistar rats. All rats were tested for survival with the afore mentioned hemodilution model (Lundgren, Bergoe and Tyssebotn 2006). They all survived surgery. After hemodilution to less than 2 g hemoglobin/100 mL blood while given 0.7 mL DDFPe/kg body weight, the rats lived for 2 hours, which was our preset requirement of a high-quality emulsion.

MODEL I: HEMORRHAGE IN ANAESTHETIZED PIGS WITH BROKEN FEMUR AND LAPAROTOMY

Most results from **Model I** pigs were reported in the written report on March 31, 2008 and verbally given at the ATACCC Conference, St. Pete's Beach, FL, August 2008.

Since March 2008, more pigs have been added to each study group. The results confirm and strengthen the conclusions from last year. Non-treated pigs had a survival rate of ~30% after suffering a fractured femur, soft tissue damage and severe blood loss. When Hextend was used as treatment in this model, the survival rate increased to 80% (8 out of 10). When the new DDFP emulsion from NuvOx Pharma was applied, the survival rate was 100% (6 out of 6) in healthy pigs.

MODEL II: HEMORRHAGE IN SEDATED PIGS - COMPARISON OF DDFPe TREATMENT AND HEXTEND TREATMENT

A model for hemorrhagic shock and bleeding in sedated, awake pigs was developed over the spring of 2008. After 3 months of negotiations with the IACUC committee at the university, we were granted approval for the protocol to conduct the experiments under semiconscious conditions. Two members of the IACUC committee supervised the bleeding procedure during the first two experiments to ensure the pigs did not suffer in any way.

The timeline for sedated pig experiments

Habituation: Habituation of the pigs lasted for 4-5 weeks to adapt them to be relaxed in a supporting sling when sedated for many hours during the hemorrhage experiment.

Day 1 (surgery): Pigs were anesthetized and surgically prepared with a limited number of arterial and venous catheters.

Day 2 or 3 (hemorrhage): Hemorrhagic shock experiments were performed.

Day 16 (necropsy): The pigs were anesthetized and physiological measurements performed, followed by euthanization, and harvesting of tissue samples.

The training/habituation program

Sixteen pathogen free neutered male pigs weighing 8-10 kg were purchased from Michal Fanning Farm, IN, and grew to ~23 kg by the time of experiment.

The pigs were trained daily by a limited number of people starting with touching and grooming. They were gradually exposed to the restraining sling and the equipment used in the experiments. For motivation and as a reward during and after each training session the pigs were given treats, such as apple pieces, until they were calm and relaxed in the sling. Whenever the pigs were in their pen, they had free access to regular food and water. After approximately 4 weeks of training the pigs were willing to stay in the sling for the duration of the experiments.

Surgical preparation - Day 1

The pigs were pre-medicated with Telazol, 1 mL /25 kg, and anesthetized for approximately one hour with Isoflurane gas and mechanically ventilated with 4 L of O₂/min. The entire surgery was performed aseptically and in a sterile surgical suite at the Laboratory Animal Facility of University at Buffalo (SUNY). Each pig was intubated with an endotracheal tube, size 5.5 to 7 mm. A small skin incision on the right side of the neck was made and the right carotid artery was located and cannulated with a catheter to facilitate bleeding during the experimental procedure. The right jugular vein was cannulated and the catheter tip located close to the right atrium to be used for infusions of se dative drugs and fluids. The catheters were tunneled under the skin and the ends taken out on the pig's back. Arterial blood was collected anaerobically for measurements of the arterial acid-base chemistry, blood gases, sodium, potassium, base excess, lactate and glucose on an ABL725 Blood Gas Analyzer (Radiometer, Copenhagen, Denmark). Blood pressures and heart rate (HR) were measured for approximately 1 hour and at least 2 arterial blood samples were taken during the procedure. The pigs were given an infusion of lactated Ringer's solution in the amount of 1 mL/min for the duration of the surgery. At the end of the surgical procedure, the catheters were closed and put into a small pocket sewn onto a fitting jacket worn by the pig to ensure that the pigs did not reach them. The catheters were flushed with heparinized fluids daily to reduce the risk of coagulation.

Hemorrhagic shock experimental procedure - Day 2 or 3

Post surgery and until time of euthanization, the pigs were checked daily by a veterinarian. The pigs were removed from their pen after given a small dose of either of two drugs, Midazolam or Diazepam i.v. and placed in a supporting sling that allowed them to keep their feet on the ground. Of the 16 pigs that had catheters implanted, 4 pigs had to be excluded from the study. One had severe lung infection (verified with bacteriology), two had clogged catheter lines (dedicated as shams for histology), and the last did not survive the hemorrhage.

When the pigs had relaxed for 15 min in the supporting sling, the arterial pressure line was connected to a Y-adaptor where one branch was connected to a pressure transducer and the other to a bleeding line (Fig 1). The central venous line was connected to another Y-adaptor with one branch connected

to a pressure (CVP) transducer and the other branch to a pump to be used for infusion of sedative drugs, plasma expander fluids and DDFPe (Figure 1).

Prior to bleeding, baseline measurements were recorded for 30 min and 3 arterial blood samples and 1 venous sample were taken and assessed. The pigs were then bled ~31.5 mL/kg over 2–6 min, reducing the blood pressure below 50 mmHg. The pigs remained calm during the entire bleeding procedure. From 30 min prior to the onset of bleeding, arterial and venous pressures, respiratory frequency (RF) and heart rate (HR) were measured continuously. The start of hemorrhage was set as time zero. For measurements of O₂ and CO₂ tensions, base excess, hemoglobin, potassium, sodium, chloride, glucose and lactate concentrations and pH, arterial blood was sampled every 5 min (~1 mL each) for the first 45 min and every 15 min thereafter, while venous blood (~1 mL) was sampled every 60 min for the entire experiment. In addition, 5 mL arterial blood was sampled every 60 min throughout the experiment for analysis of urea, creatinine and DDFP concentrations. As a final result, blood sampling ended at roughly 10 mL/hr for the duration of the experiment.

Hemorrhagic shock was allowed to develop from the onset of bleeding until ~63 min mark, where after treatment with either DDFPe (0.6 mL/kg) or Hextend (7 mL/kg) was infused over the next 30 min. After the DDFPe infusion, 7 mL of venous blood was sampled to clean the DDFPe out of the venous line. An identical volume was sampled from the Hextend pigs.

After a period of 3 hours, 500 mL of Hespan (6% hetastarch in a 0.9% NaCl solution; H.Braun Medical Inc., Irvine, CA) was infused over 60 min in all pigs and the wounds closed using local anesthetics, after which the pigs were returned to their pen.

Physiological measurements and euthanasia - Day 11 or 14

A Hextend pig that the veterinarian required to be euthanized on day 11 had an extremely poor condition without any detectable reason. It was unable to feed or stand up. All the other pigs were apparently healthy, eating and drinking and lived until Day 14, when they were euthanized as planned.

All pigs were anesthetized with Isoflurane after a standard premedication of Telazol, 1 mL/25 kg. All pigs had new catheters implanted in the right femoral artery and the left jugular vein for arterial and venous pressure measurements. A Foley catheter was implanted into the urinary bladder through a small incision in the lower abdominal wall.

After a stabilization period of 45 min, at least 3 parallel arterial and venous blood samples as well as urine samples were taken in order to assess kidney function by calculation of urea and creatinine clearance, as well as estimation of renal blood flow.

From the arterial and venous blood samples, O₂ and CO₂ tensions, base excess, hemoglobin, potassium, sodium, chloride, glucose, urea, creatinine, lactate concentrations and pH were determined. The measurements were performed over 1 hour after which a light hemodilution, reducing hemoglobin concentration by 30%, was performed before the pigs were euthanized with a lethal dose of pentobarbital (Fatal Plus, Vortech Pharmaceutical, Dearborn, MI). Tissue samples were harvested from the following organs: heart, lungs, cerebrum, cerebellum, brain stem and spinal cord,

spleen, liver, stomach, small and large intestines, kidneys, and adrenals. The tissue samples were stored in 4% buffered formaldehyde before processed for light microscopy. So far, only histopathology of pig kidney has been provided by Professor Peter Nickerson, PhD, SUNY at Buffalo. The following groups of pigs were examined: Control (sham operated), Hextend and DDFPe. Following the fixation with buffered formaldehyde, kidneys were processed by conventional techniques for light microscopy; 4 µm sections were cut and stained with hematoxylin and eosin.

MODEL III - RAPID INFUSIONS OF DDFPe AND HEXTEND

Six pigs, weighing 20-25 kg, anesthetized with pentobarbital sodium (25 mg/kg), were tested for their tolerance of increased infusion rate of DDFPe or Hextend treatments. The pigs were instrumented as was done for the broken bone/hemorrhagic model (**Model I**) (Figure 11) reported in March 2008. The leg was broken 5 min before the bleeding was started. A bleeding of ~ 28 mL/kg was performed over 18 min in all pigs. The six pigs were randomly divided into two groups treated with an infusion of either Hextend (7 mL/kg) or DDFPe (0.6 mL/kg) over a 15 min time period.

Oxygen consumption and CO₂ production were measured by an airflow meter, and mass spectrometer measurements of O₂ and CO₂ in expired gas, were also taken. Results were converted to mL/kg (STDPD). Cardiac output was measured by a Millar blood flow catheter (Millar Instruments, Houston, TX) placed in the left ventricle. Tissue electrodes from Radiometer (Copenhagen, Denmark) were used for tissue O₂ and CO₂ measurements.

STATISTICS AND CALCULATIONS

All values are given as means ± SEM (standard error of the mean). P-values of 0.05 or less were considered significant. Creatinine and urea clearances were calculated from plasma and urine concentrations and urine flow on the final day of the experiments. Clearance of creatinine and glomerular filtration rate (GFR) are believed to be equal. Assuming the filtration fraction to be 20%, the renal plasma flow and blood flow were calculated from the measured creatinine clearance (GFR) and adjusted by the hematocrit. Blood volume was calculated from the fall in hemoglobin after infusion of known amounts of Hextend (Model II and III) and Hespan (Model II). Shock index was calculated as heart rate divided by mean arterial pressure and cardiac work index as heart rate times systolic arterial pressure.

RESULTS

MODEL II - SEDATED PIGS GIVEN DDFPe OR HEXTEND TREATMENTS

The training/ habituation

All sixteen pigs were successfully trained to stand relaxed in the supporting sling for up to two hours.

Day 1 - Physiological measurements during anesthesia

Arterial pressure, CVP, HR, RF and arterial blood parameters were similar and within normal range in all sixteen pigs post surgery (Figure 2 to 10). They all recovered from the Isoflurane anesthesia within 15 min after surgery was completed and started eating immediately after being returned to the pen.

Day 2 - Hemorrhagic shock development and treatments during light sedation

The arterial pressures, CVP, HR and RF values prior to bleeding were similar between the two groups of animals (Figure 2 to 5). Blood samples taken before the hemorrhage and Iso had identical compositions, as shown in Figure 7 to 10. The differences in values from 2 days before were most likely caused by mechanical ventilation, O₂ breathing, and Isoflurane anesthesia.

The arterial pressures fell similarly in both groups during bleeding (Figure 2). While hemorrhage increased the HR during bleeding of pentobarbital anesthetized pigs as previously demonstrated (annual report March 2008), HR fell during bleeding of the semiconscious pigs (Figure 3). Both groups of pigs were bled identically (31.9 ± 0.5 mL/kg in 26 ± 0 min), the shock developed similarly until the treatment began 63 min after the onset of bleeding (Figures 2 to 6), and the blood composition developed equally (Figures 7 to 10).

HEXTEND TREATED PIGS

A few minutes after the Hextend infusion was initiated, the arterial pressures started to increase gradually as demonstrated in Figure 2, upper panel, and stabilized around 100 mmHg during the rest of the period before the plasma expander (Hespan) was initiated. A smaller increase was observed during the plasma expander infusion. The HR fell after the Hextend infusion, but increased during the infusion of plasma expander (Figure 2, upper panel). The respiratory frequency increased during bleeding, then started to fall transiently before a significant and a marked increase (~40%) occurred during Hextend infusion. The rate again fell at the end of the infusion, followed by another increase after start of plasma expander infusion (Figure 4, upper panel).

DDFPe TREATED PIGS

The arterial pressure showed a rapid, transient increase during the first 10 min of DDFPe infusion. After the treatment, arterial pressure continued to increase gradually during the whole period before Hespan infusion. The HR was unchanged during bleeding and before start of DDFPe treatment, increased slightly during treatment, gradually fell during the period before Hespan treatment, and increased gradually afterwards (Figure 3, lower panel). The RF remained mostly unchanged during bleeding and treatment and the period before Hespan was given, but fell abruptly during this infusion. At the end of Hespan infusion, the RF increased steeply (Figure 4, lower panel).

COMPARISON BETWEEN THE TWO GROUPS OF ANIMALS

The shock index (Figure 5) and cardiac work index (Figure 6) described similar patterns and were not statistically different in the two groups. The dilution effect of Hextend on arterial blood is shown in Figure 7, resulting in an arterial oxygen content significantly higher ($P<0.01$) in the DDFP group than the Hextend treated group (Figure 8). Since the O₂ content is calculated from oxyhemoglobin

concentration and oxygen tension measured by the ABL 725, the O₂ content values in the DDFPe group (during and after treatment) is most likely a minimum value since the machine might not be measuring the O₂ in the microbubbles. Concentrations of lactate, glucose, potassium and base-access were similar during the experiment in the two groups of animals (Figure 10).

When returned to the pen after the bleeding experiment, all DDFPe treated pigs immediately started to eat and drink, while the Hextend treated pigs primarily slept.

Day 14 – Physiological measurements, euthanasia, and necropsy

By the day of necropsy, most of the pigs had developed small, local, superficial infections in the wound surfaces on the neck. As mentioned previously, one of the Hextend pigs required to be sacrificed the 11th day after hemorrhage, as it had not gained any weight, was inactive, and was in very poor condition. There was no indication of infection or temperature increase. All other pigs in both groups had gained weight, 0.3 ± 0.1 kg/day (Hextend) and 0.4 ± 0.1 kg/day (DDFPe), were in good condition, and lived for the 14 days as required. On the day of necropsy, the arterial and central venous pressures as well as blood compositions were similar to the values during identical anesthesia on Day 1. The pressures and blood composition values were similar in both treatment groups and similar to the values measured in anesthesia prior to the bleeding experiment. The creatinine clearance was similar in both groups (182 ± 13 and 184 ± 11 mL/min, DDFPe and Hextend group, respectively), while the protein clearance was significantly higher in the Hextend group than the DDFPe group (0.58 ± 0.28 and 0.05 ± 0.01 mL/min, P<0.05, respectively) indicating significantly more proteins were excreted, possibly due to glomerular membrane leakage. Renal blood flows calculated from creatinine clearance were 1253 ± 88 mL/min for the DDFPe group and 1257 ± 81 mL/min for the Hextend group. Only one animal from the DDFPe group had detectable concentration of DDFP in the lung. No animals had DDFP detected in the fat.

During the necropsy, pale, wedge-shaped areas within the cortex and the outer medulla of the kidneys were regularly found in the Hextend treated pigs, but not in the DDFPe treated pigs.

By light microscopy, the histopathology of kidneys, including the glomeruli in the cortex from the DDFPe group, was virtually identical to the control group (sham-operated). Glomeruli in the Hextend group showed a small amount of vacuolation that corresponds to prominent capillaries. Tubules throughout the cortex and medulla were similar among all three groups of animals.

MODEL III - INCREASED INFUSION RATE DURING DDFPe OR HEXTEND TREATMENT

These pigs were anesthetized with pentobarbital that reduced the arterial O₂ and increased the CO₂ tensions compared to the sedated pigs in the previously described series (Model II). Otherwise the results are comparable between sedated and anesthetized pigs. Only 1 pig from each treatment group survived for the required 6 hours (Figure 12). Two Hextend treated pigs died at a mean of 130 min after start of bleeding due to pulmonary distress, reduced arterial O₂ and increased CO₂ tensions. The two pigs that died after DDFPe infusion survived both for a marked longer time (mean: 233 min) than the Hextend pigs that died. One of the DDFPe treated pigs that died had an anomaly with a considerable channel in the septum between the two atriums of the heart mixing the oxygenated and venous blood (verified by high O₂ tensions in venous blood samples). This circulatory pattern could

possibly reduce the loading of O₂ into the microbubbles of DDFP. Results from the measurements are shown in Figures 12 to 19.

During deep anesthesia, it was remarkable that when the femur was fractured, the O₂ consumption and CO₂ production immediately fell in all animals by 25-30 % before the bleeding started 5 min later (Figure 14 and 15). Similarly, minute ventilation fell after the bone break and started to increase during bleeding. During treatment, only Hextend increased the minute ventilation by 42% while DDFPe treatment increased the minute ventilation by only 4 %. The work of breathing/min (calculated as: (tidal volume)x(pressure changes during the breathing cycle) x(breathing frequency)) increased by 67% during Hextend treatment while it only increased by 9% during the DDFP treatment.

DISCUSSION

The fundamental goal of this study is to elucidate the most effective treatment of hemorrhagic shock in a field-applicable setting. There is no obvious difference in the outcome of the two treatment modalities, except that one of the sedated Hextend treated pigs in Model II required early euthanasia and other pigs during Hextend infusion developed respiratory distress (Model I under Goal 3, Model II and Model III). Treatments with the microbubbles created from DDFP emulsion was, in our view, the best of the two options since blood pressure steadily increased during and after the infusion, then remained stable for several hours (Model I under Goal 3 and Model II). In addition, the animals seemed healthier during the recovery after the experiment, eating better, no death occurred over 14 days, and no renal changes were observed microscopically (Model II). Perhaps a combination of DDFP microbubbles and a small amount of Hextend could give a better outcome, although this was not evaluated in the present study. Additional positive observations are that DDFP emulsion does not need refrigeration during storage, the shelf life is long (16 months, so far, for the emulsion produced by NuvOx LLC in February 2008; 10 years for the product made by Sonus Pharmaceuticals, Inc., Bothell, WA), the difficulties of matching donor/recipient blood type is prevented, and the volume is so small that an individual soldier could bring his/her own sterile bottle of DDFPe solution into the field. Indeed, Hextend used as a resuscitation fluid is 12 times as voluminous as DDFPe, putting an extra burden to the paramedic in the field (500 mL given to a soldier). Additional disadvantages of Hextend discovered in this study include lower weight gain than normal, and occasional deaths, as only 84% of the animals survived for 14 days (Model II).

One of the aims in this study was to increase the infusion rate of a full dose of Hextend and DDFPe to be given in 15 min (Model III). This was possible in both types of treatment, but only one out of three pigs in each group survived for 6 hours which was our requirement for the study. The two Hextend treated pigs died after an average of 130 min, whereas the two DDFPe treated pigs died after about 240 min, even with one of the latter pigs having a significant hole in the cardiac septum, possibly influencing the survival time. The two Hextend treated pigs died with pulmonary distress, reduced arterial O₂, and increased CO₂ tensions suggest that the increased infusion rate caused a detrimental overload of fluid.

KEY RESEARCH ACCOMPLISHMENTS

- New DDFP emulsion (NVP-108, NuvOx Pharma, LLC) is produced in laboratory scale.
- The emulsion appears to be stable after 16 months.
- The emulsion functions well when tested in both oxygen depleted rats and pigs.
- Three hemorrhagic shock models in pig have been successfully developed and tested.
- Treatment with 0.6 mL/kg DDFP emulsion demonstrates excellent outcome in pigs after hemorrhagic shock.
- Hextend infusion of 7 mL/kg showed some dangerous side effects and demonstrated lesser survival in hemorrhagic shock than infusion of DDFPe.
- The oxygen content in arterial blood was kept higher in DDFPe treated pigs than in Hextend treated pigs providing more O₂ to the tissue if blood flow was similarly maintained.
- Kidney physiology and histology was normal in the DDFPe treated and sham-operated pigs.
- The Hextend treated surviving pigs lost proteins in urine on necropsy day, most likely caused by membrane destruction in the glomeruli.
- Histology of the kidneys in Hextend treated pigs showed vacuolization in the glomeruli.
- Increased rate of Hextend infusion provoked symptoms of pulmonary distress.
- The higher infusion rate gave longer survival time in DDFPe treated pigs compared to Hextend treated pigs.
- Treatment with DDFP emulsion remains the most promising treatment module for Hemorrhagic shock.

REPORTABLE OUTCOME

Tyssebotn, I, GW Bergoe: Treatment of Hemorrhagic Shock: Comparison between 0.5 mL/kg DDFP emulsion (microbubbles) and 7 mL/kg Hextend. ATACCC Conference, St. Pete Beach, Florida, 11th August 2008.

Tyssebotn, I, GW Bergoe; Treatment of Hemorrhagic Shock: Comparison between 0.6 mL/kg DDFP emulsion (microbubbles) and 7 mL/kg Hextend in semi conscious pigs. ATACCC Conference, St. Pete Beach, Florida, to be held 10th-13th August, 2009.

CONCLUSIONS

1. A new and stable DDFP emulsion is available.
2. Pigs in hemorrhagic shock can be successfully resuscitated with an extremely small dose of DDFP emulsion (15 mL/25 kg).
3. The volume of Hextend used is twelve times the volume of DDFPe.
4. DDFPe as resuscitation fluid seems more effective than Hextend

5. Low volume resuscitation can be accomplished with DDFP emulsion.
6. If pertinent, a wounded soldier in the field can be treated with about 45 mL of DDFPe emulsion.

SO WHAT

Since the pigs survived after one dose of DDFPe or Hextend, and the time frame for experiments was too short a second dose was not attempted.

The older DDFP emulsion (Echogen) produced by Sonus Pharmaceuticals Inc. has been tested in roughly 2500 humans, volunteers and patients, and approved for ultrasound imaging in humans in Europe. Sonus was not able to get Echogen approved by FDA. NuvOx Pharma, LLC (Tucson, AZ) has been able to make a replica of Echogen that has been used in the present study. This replica is working equally well as the predecessor. NuvOx Pharma, LLC is a small company with limited resources and will not, without funding, be able to get the product approved by FDA and clinically tested.

Treatment with DDFP emulsion is still the most promising resuscitation fluid for hemorrhagic shock, but more research has to be performed to evaluate the safety of the DDFP emulsion.

PROBLEMS MET AND SOLVED DURING THE PROJECT

The work planned under Aim 1 in the original proposal, would have brought the preparation to maturity in terms of fulfilling the sponsor's requirements under Product Considerations (page 3, Supplement 1) as well as with regards to toxicology and GMP. This was not accomplished since ImaRx Pharmaceuticals, Inc. met financial problems. They closed down the production and later abandoned the project. Therefore, at the end of the grant period (i.e. end of year 2), the product from the new company NuvOx Pharma, LLC was still not ready for an FDA IND permit, even as a successful outcome of work under Aim 2 (in the original proposal) has been demonstrated.

Change of PI created some delays in the middle of the grant period.

One of the key researchers became seriously ill and was on sick leave for three months.

Negotiations between NuvOx Pharma, LLC and the University of Buffalo took several months, but discrepancies were solved in January 2008 (see Appendices).

In the spring of 2008, 3-4 months of negotiations and modifications of the research protocol were invested before the IACUC committee at the University was willing to approve the protocol for bleeding of habituated, sedated pigs.

Shortage of a technician in our lab lasted for more than 6 months and therefore delayed processing of data.

The histology of tissue specimens harvested during necropsy has not yet been fully processed due to shortage of personnel in the histology lab of Dr. Peter Nickerson. The kidney specimens were

however processed a week ago and the results included in this report. The remaining histology mentioned is still pending.

REFERENCES

Burkard ME, Van Liew, HD. Oxygen transport to tissue by persistent bubbles: Theory and simulations. *J. Appl. Physiol.* **77**, 2874-2878, 1994.

Lundgren C, Bergoe G, Tyssebotn I. Intravascular fluorocarbon-stabilized microbubbles protect against fatal anemia in rats. *Artificial Cells, Blood Substitutes, and Biotechnology, and International Journal.* **34**, 473-486, 2006.

APPENDICES

1 - FIGURES WITH LEGENDS

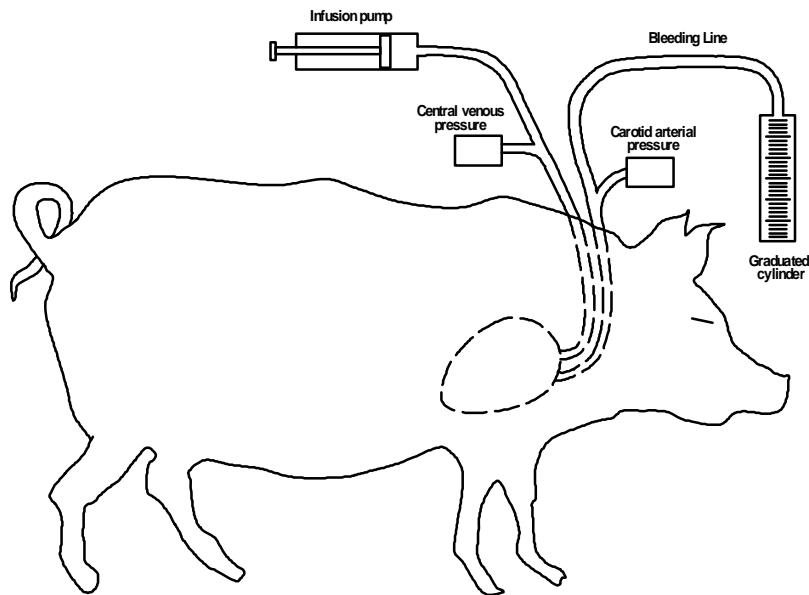


Figure 1: A schematic drawing of a sedated pig positioned in a sling prepared for experimental hemorrhagic shock (Model II). Catheters for bleeding and arterial pressure recording from aorta, and infusion of sedative and treatment in a vein (vena jugularis) are shown. The pigs were bled 31.9 ± 0.5 mL/kg body weight during 26 ± 0 min. When all the blood samples were added at the end of the experimental day, 344 ± 0 min after start of bleeding, the animals had lost 35.3 ± 0.8 mL/kg. All values are means \pm SE. The experiments were performed under heavy scrutiny of the veterinarian staff.

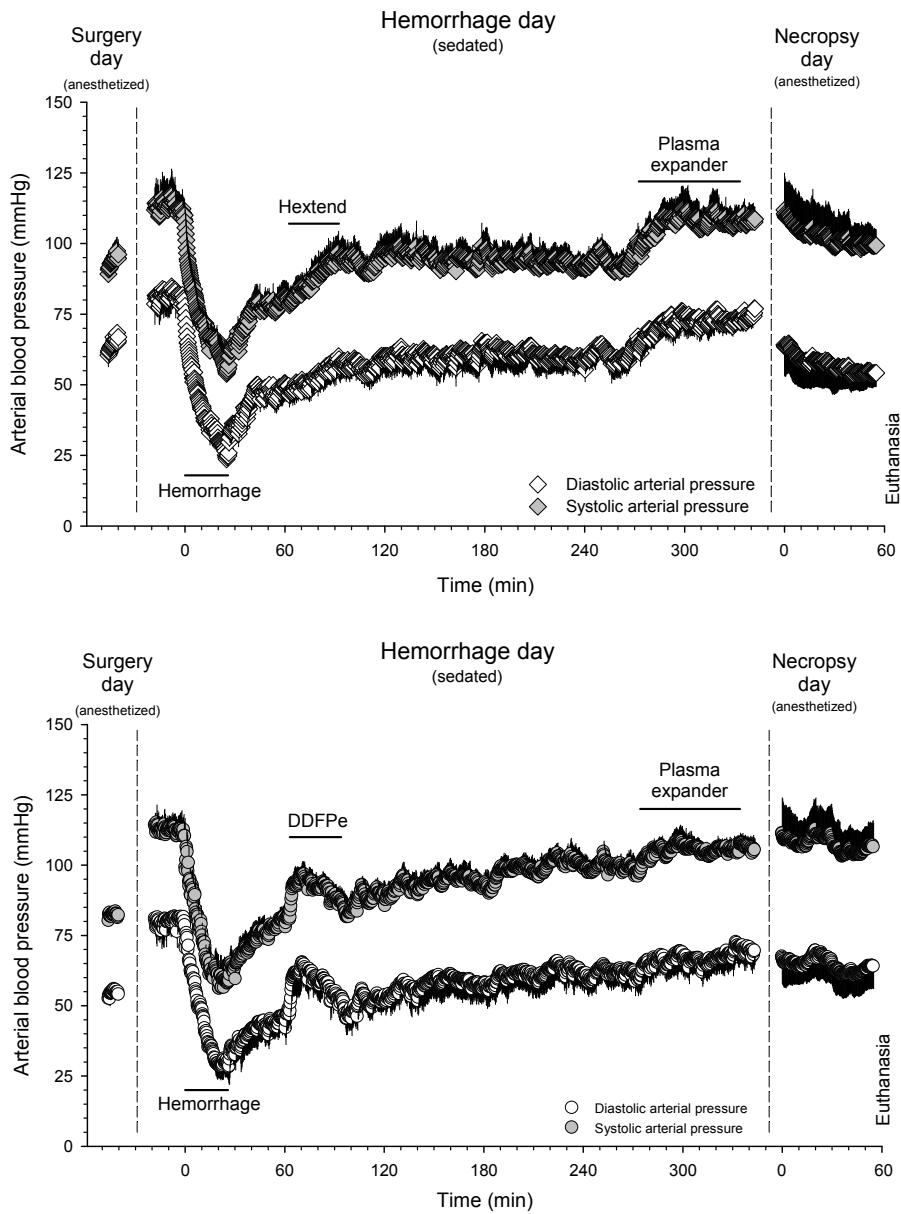


Figure 2: The figure shows the timeline and events during hemorrhagic shock experiments in sedated pigs. This includes the surgery day 2 to 3 days before the hemorrhage day, and the necropsy day 11 to 14 days after the severe blood loss ($31.9 \pm 0.5 \text{ mL/kg}$). Systolic and diastolic arterial pressure are shown in two groups of pigs ($n=6$ in each). About 35 min after the hemorrhage the pigs were treated over 30 min with either 7 mL/kg of Hextend (6% hetastarch in lactated electrolyte injection) (upper panel) or 0.6 mL/kg of DDFPe (dodecafluoropentane emulsion) (lower panel). Which of the treatments to be applied was chosen by coin toss 10 min before the infusion was to start. Three hours after the treatment had finished, a second infusion of plasma expander (500 mL of Hespan (6% hetastarch in 0.9% sodium chloride injection) during 60 min) was given to all animals. The catheters were closed and the wounds sutured in local anesthesia and heavy sedation about 10 min after the end of the last infusion.

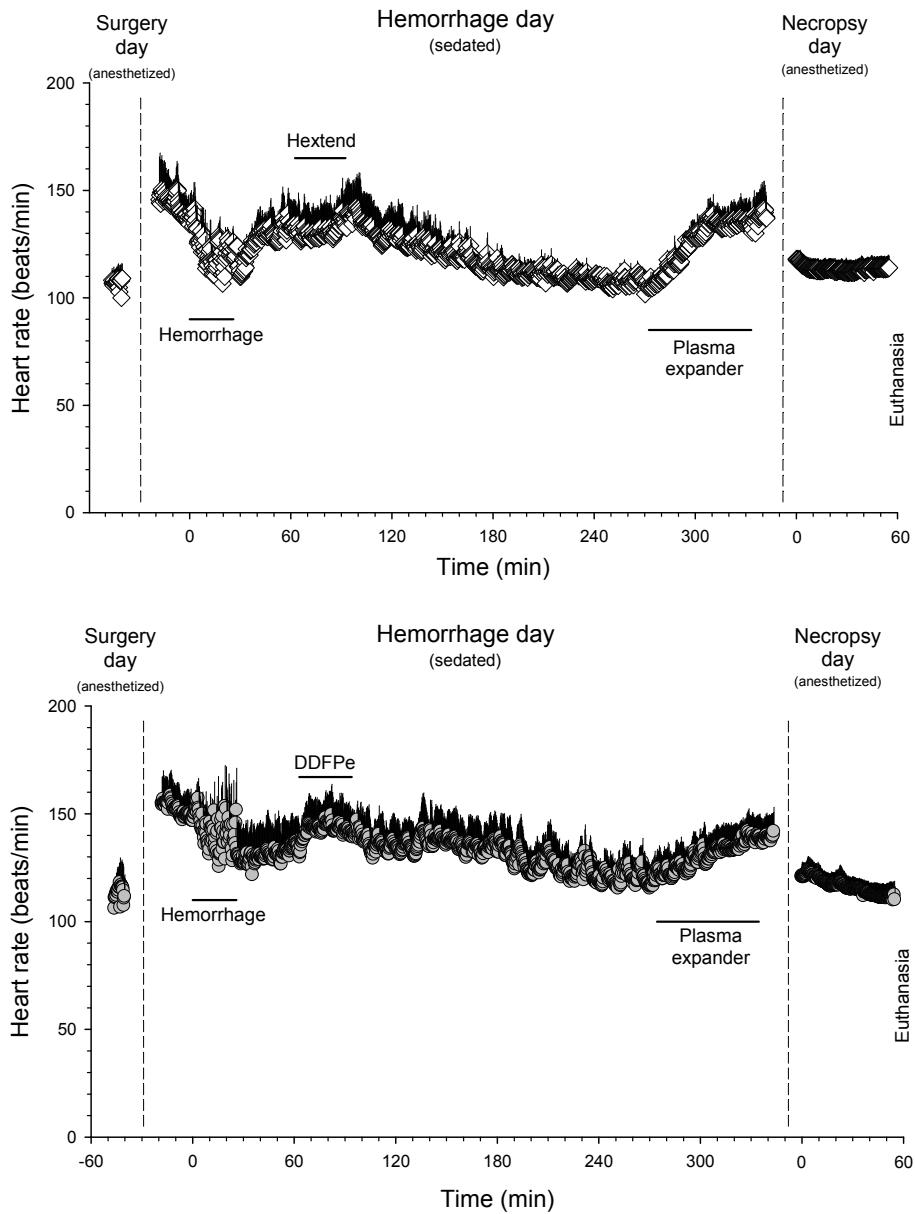


Figure 3: The effects on heart rate of hemorrhage and treatments thereof are demonstrated in two groups of sedated pigs. Hextend ($n=6$) treated pigs are shown in upper panel and DDFPe ($n=6$) treated ones in lower panel. For other details see Figure 1 and 2.

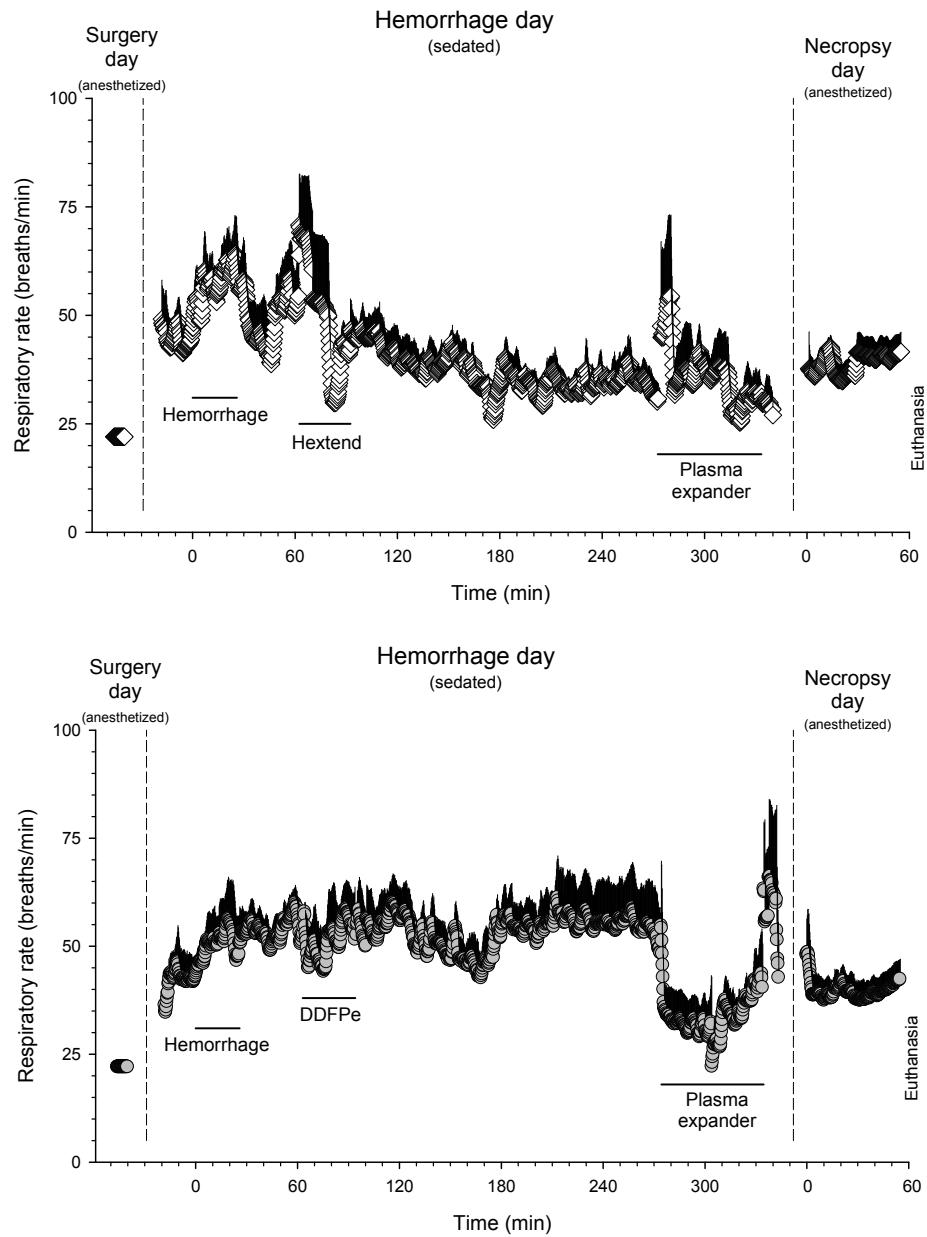


Figure 4: Respiratory rates are shown in two groups of sedated pigs during hemorrhage and treatments thereof. He xtend ($n=6$) treated pigs are shown in upper panel and DDFPe ($n=6$) treated ones in lower panel. For other details see Figure 1 and 2.

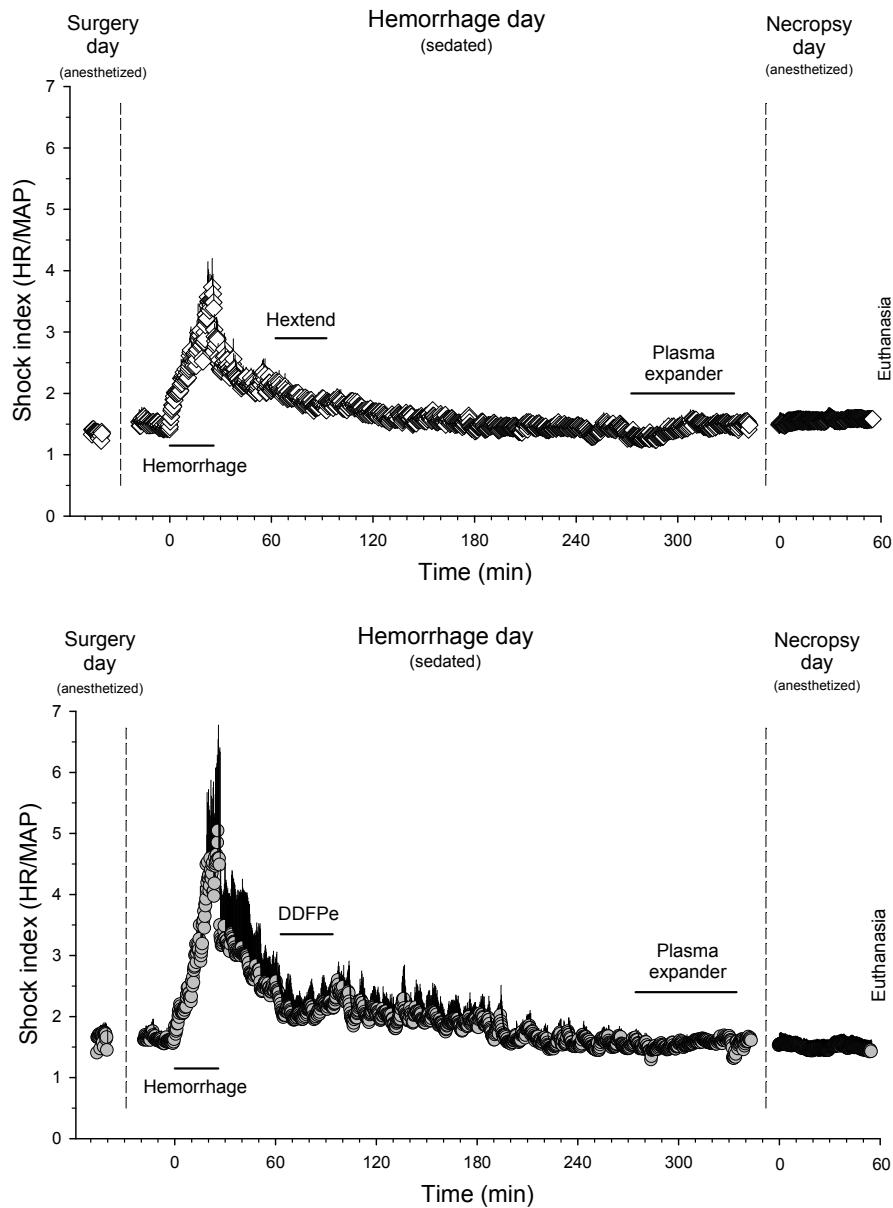


Figure 5: The effect of hemorrhage on shock indexes, calculated as heart rate divided by mean arterial pressure, are demonstrated in two groups of sedated pigs. Hextend ($n=6$) treated pigs are shown in upper panel and DDFPe ($n=6$) treated ones in lower panel. For other details see Figure 1 and 2.

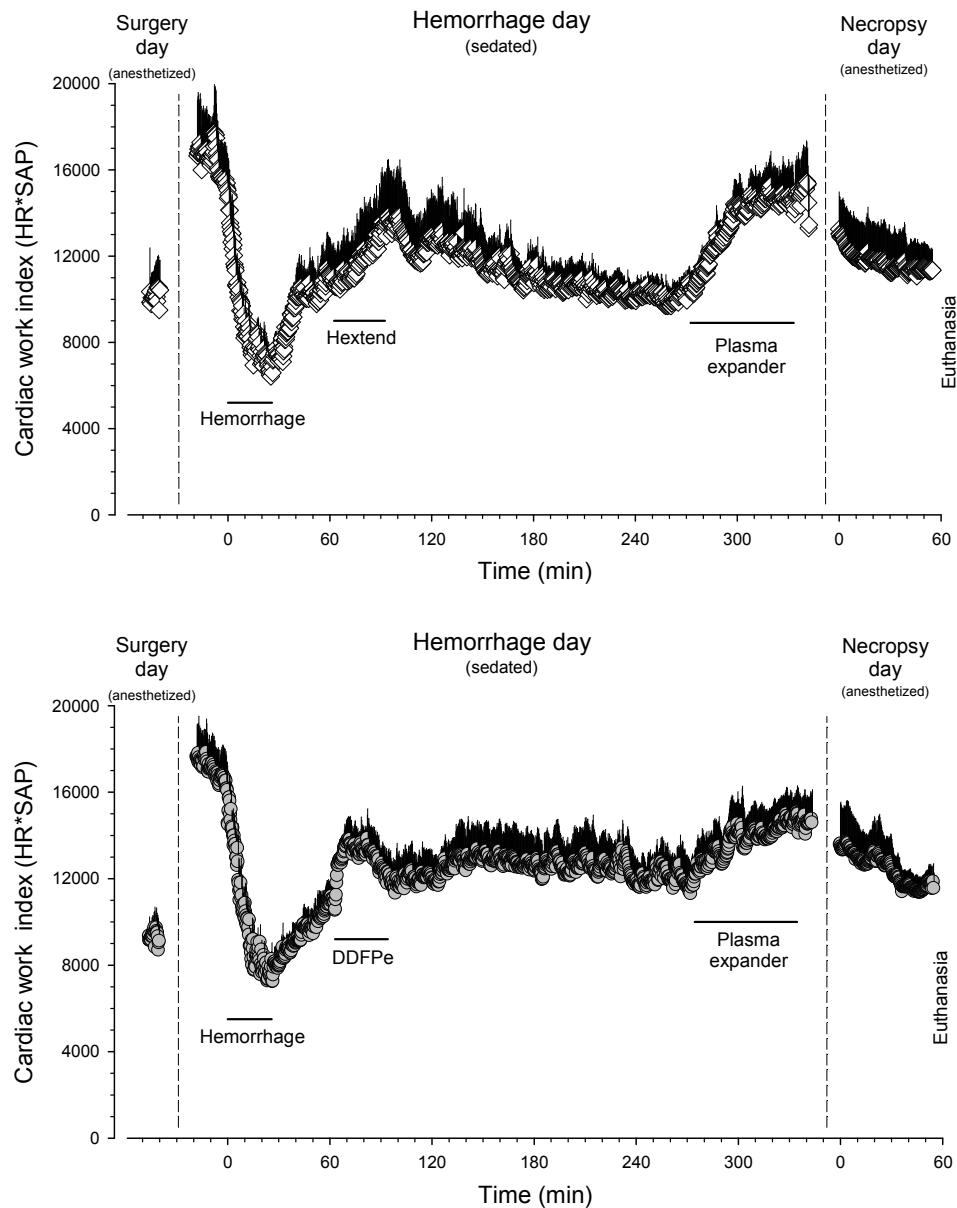


Figure 6: The effects on cardiac work indexes, calculated as heart rate times systolic arterial pressure, of hemorrhage and treatments thereof are demonstrated in two groups of sedated pigs. Hextend ($n=6$) treated pigs are shown in upper panel and DDFPe ($n=6$) treated ones in lower panel. For other details see Figure 1 and 2.

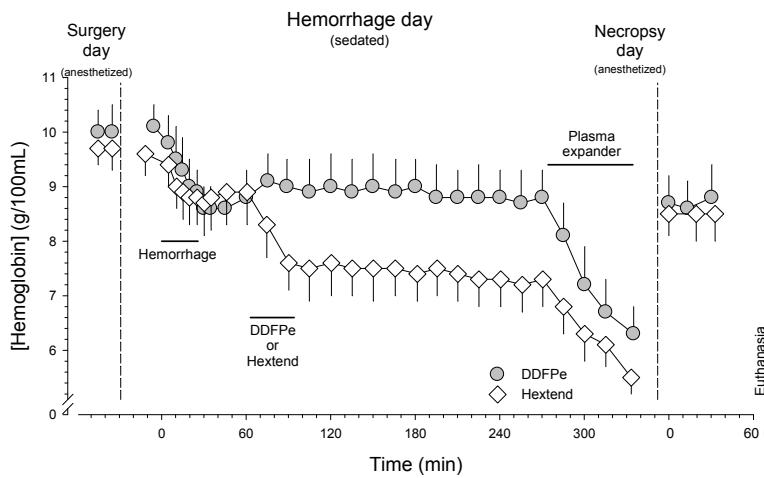


Figure 7: The variations in arterial blood hemoglobin concentration during severe hemorrhage and treatment here are shown in two groups of sedated pigs. Hextend ($n=6$) treated pigs are presented as white diamonds while DDFPe ($n=6$) treated ones are presented as grey circles. Note the significant fall in hemoglobin concentration after the start of Hextend infusion ($P<0.01$). For other details see Figure 1 and 2.

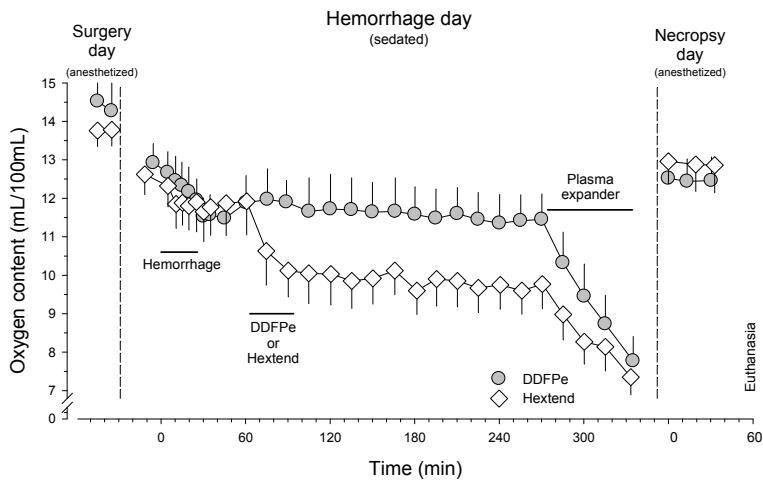


Figure 8: The effects on arterial blood oxygen content of hemorrhage and treatments thereof are shown in two groups of sedated pigs. Hextend ($n=6$) treated pigs are presented as white diamonds while DDFPe ($n=6$) treated ones are presented as grey circles. There is a significant difference between the two groups after treatment ($P<0.01$). The oxygen content in the DDFPe pigs is most likely underestimated during the next hours after treatment starts. The "blood gas" machine used for measurements is able to measure oxy-hemoglobin (done at 2 ATA) and oxygen tension in plasma, but not likely able to measure oxygen content in the microbubbles. For other details see Figure 1 and 2.

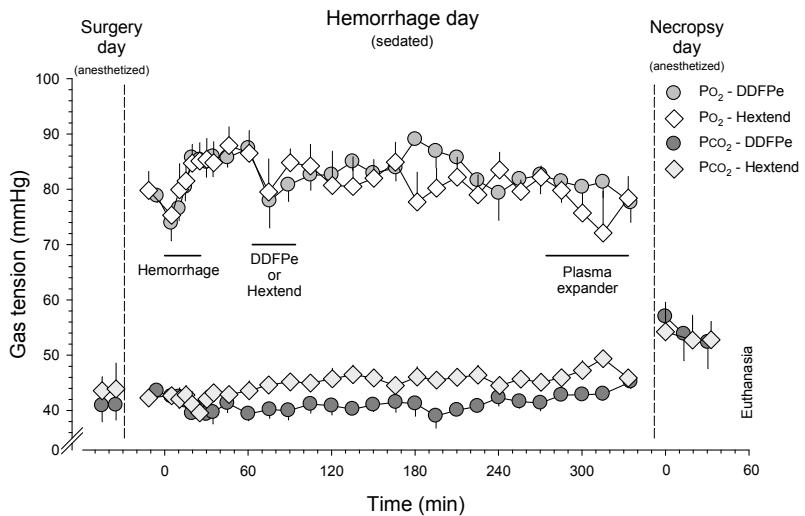


Figure 9: Arterial oxygen and car bon dioxide tensions shown in two groups of sedated pigs during severe hemorrhage and treatment there of. Gas tensions for He xtend ($n=6$) treated pigs are presented as diamonds while DDFPe ($n=6$) treated ones ar e presented as circles. For other d etails see Figure 1 and 2.

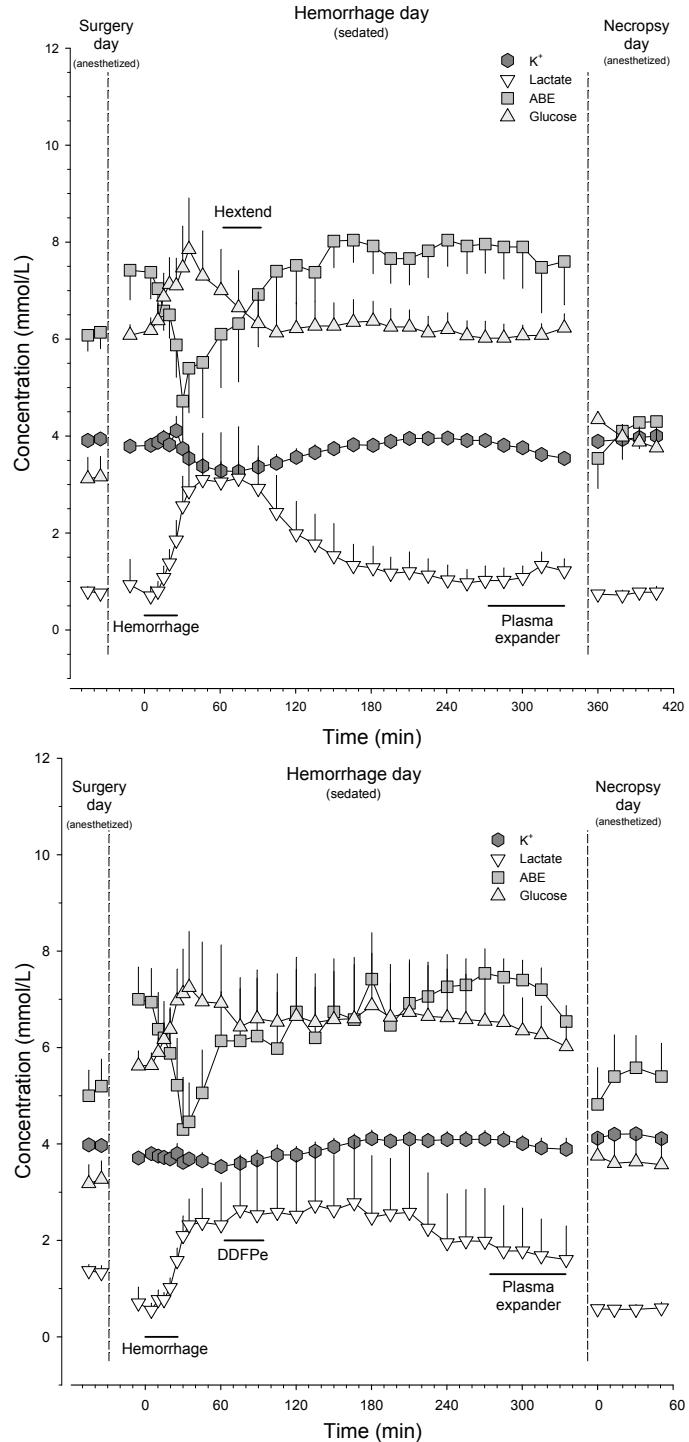


Figure 10: The effects on potassium, glucose, lactate and base-access concentration s of hemorrhage and treatments there of are shown in two groups of sedated pigs. Hextend (n=6) treated pigs are shown in upper panel and DDFPe (n=6) treated ones in lower panel. For other details see Figure 1 and 2.

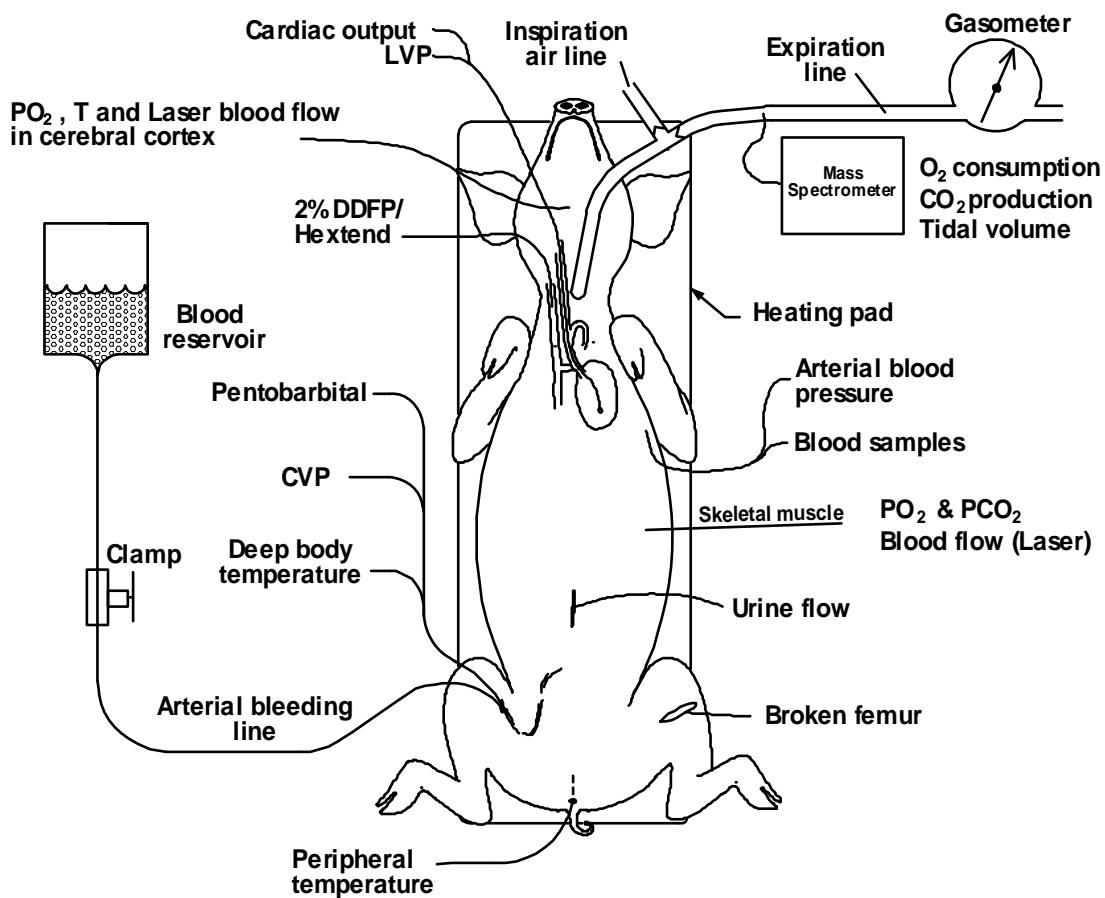


Figure 11: A schematic drawing of an anesthetized pig in supine position prepared for experimental hemorrhagic shock (Model III). Catheters for bleeding and infusion are shown as well as connections to different recording equipments. These pigs had lost $33+/-1\text{ mL}/\text{kg}$ blood at the end of the experiments.

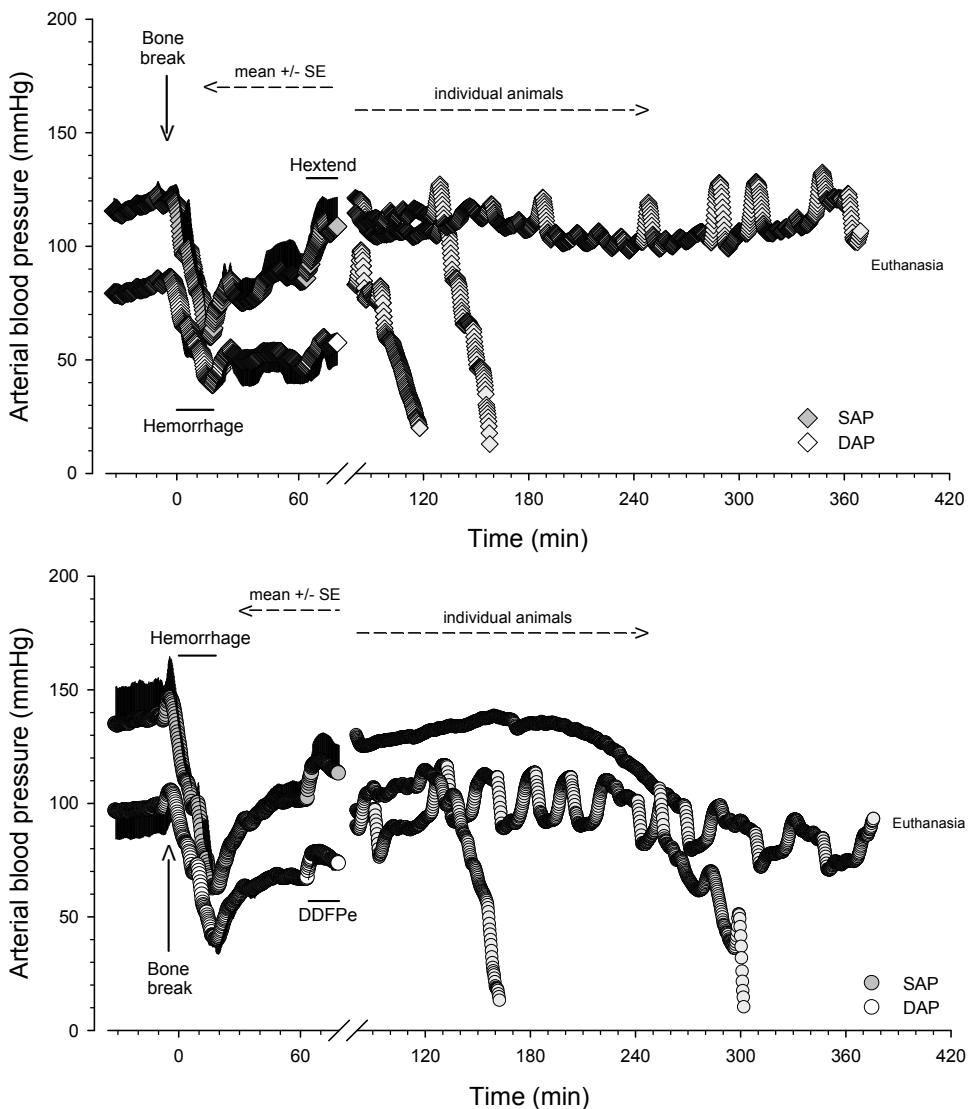


Figure 12: The figure displays the effects of bone fracture and hemorrhage on arterial blood pressures in two groups of pentobarbital anesthetized pigs ($n=3$ in each) weighing 25.6 ± 0.9 kg. The femur was broken 5 min prior to hemorrhage. Within 18 min the pigs bled 28.1 ± 0.0 mL/kg. Treatment, lasting 15 min, was started 45 min post hemorrhage. Upper panel shows the effect of Hextend infusion (7 mL/kg) and lower panel the effect of DDFPe (0.6 mL/kg). Until the end of treatment both panels displays SAP (systolic arterial pressure) and DAP (diastolic arterial pressure) as mean \pm SE, after treatment however, only SAP are shown for individual pigs. The mean survival time of the two Hextend pigs that died within 6 hrs of hemorrhage was 123 min, while the mean survival time for the two DDFPe pigs that died was 233 min. Note: One of these DDFPe pigs had a hole between left and right side of the heart ("arterial bleed" drawn from the right atrium, the hole found during necropsy). This kind of anatomical shunt has previously been noticed to reduce the survival time, independent of treatment modality in our shock pigs, but always with DDFPe pigs living the longest. Also note the instability in the pressure recordings from one animal in each group.

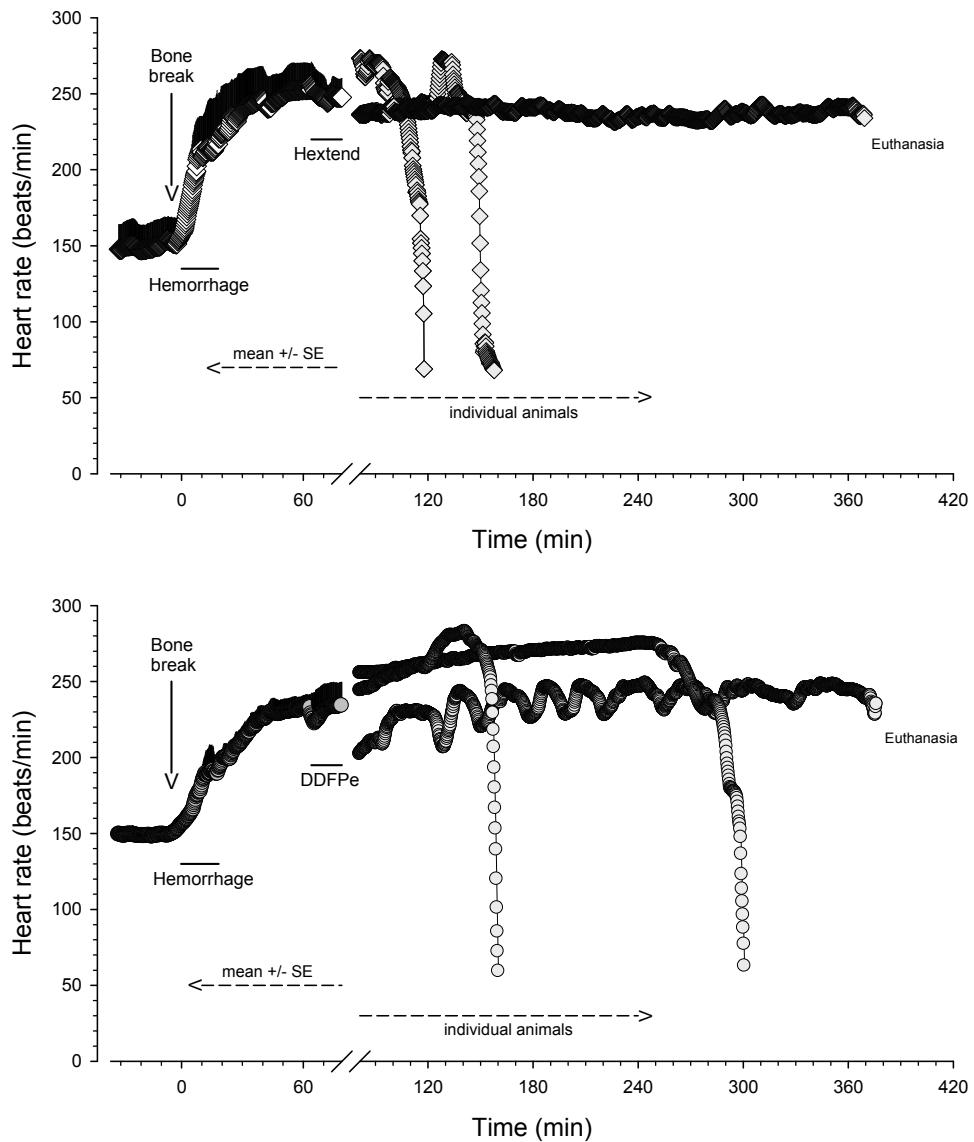


Figure 13: The effects of bone fracture and hemorrhage on heart rate in two groups of pentobarbital anesthetized pigs are displayed in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For other details see Figure 11.

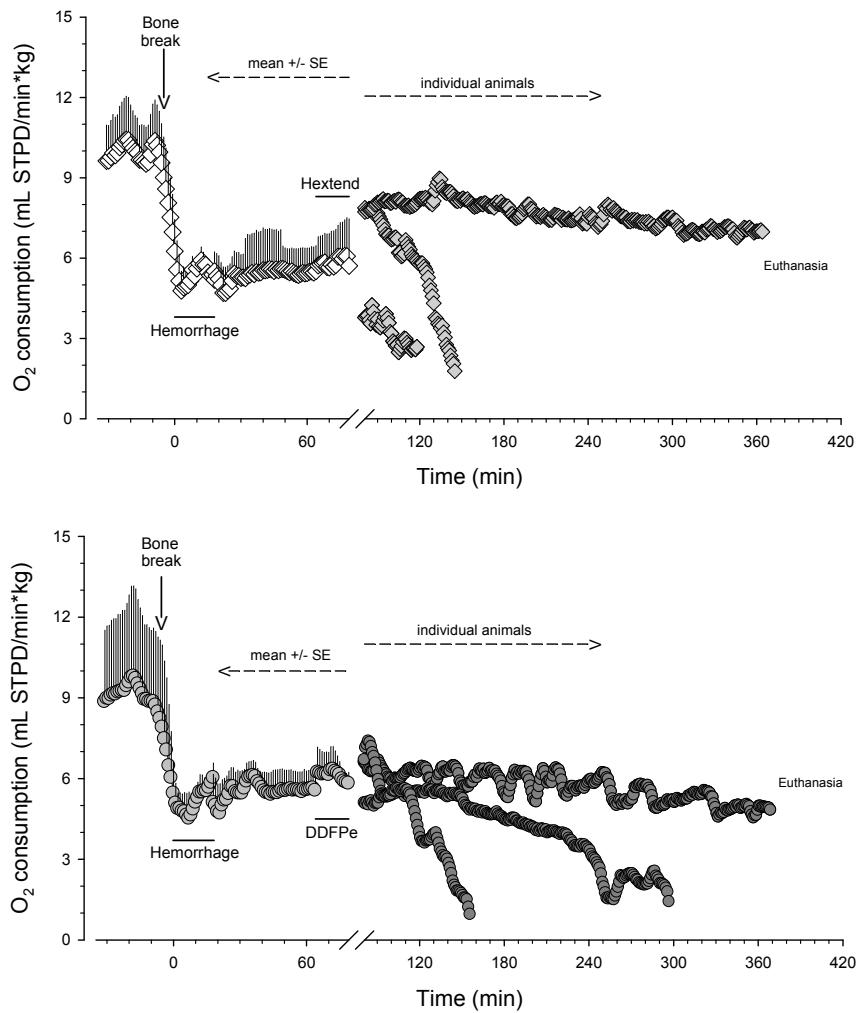


Figure 14: Effects of bone fracture and hemorrhage on oxygen consumption in two groups of pentobarbital anesthetized pigs are displayed in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). Note the abrupt fall in O₂ consumption immediately after femur was broken and before the hemorrhage was started. For other details see Figure 11.

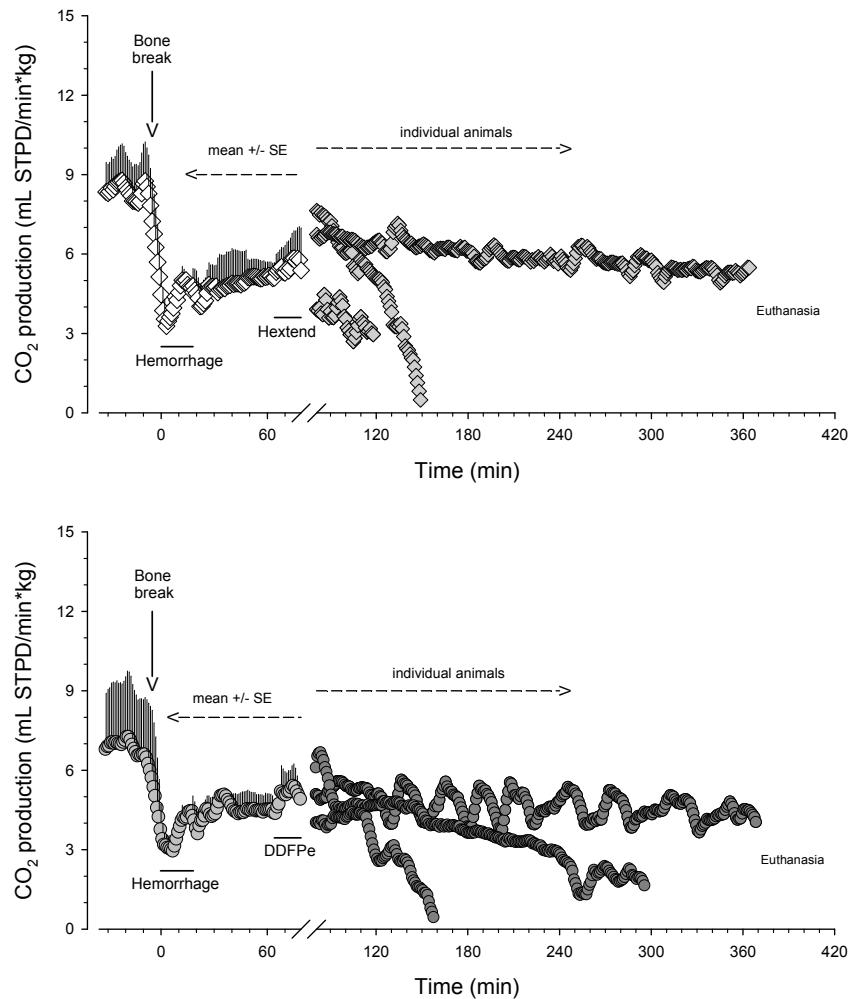


Figure 15: Changes in carbon dioxide (CO_2) production after bone fracture and hemorrhage in two groups of pentobarbital anesthetized pigs are presented in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel shows the effect of DDFPe treatment (0.6 mL/kg). Note the abrupt fall in CO_2 production immediately after femur was broken and before the hemorrhage was started. For further details see Figure 11.

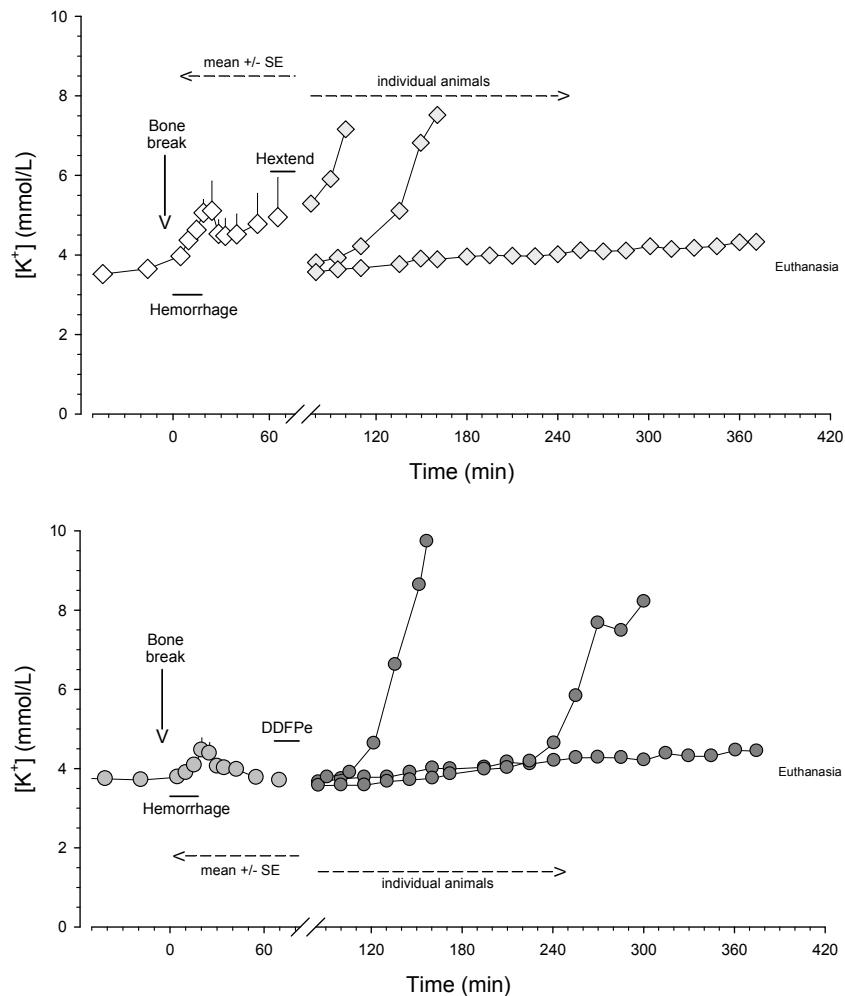


Figure 16: Changes in arterial blood potassium concentrations after bone fracture and hemorrhage in two groups of pentobarbital anesthetized pigs are demonstrated in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For additional details see Figure 11.

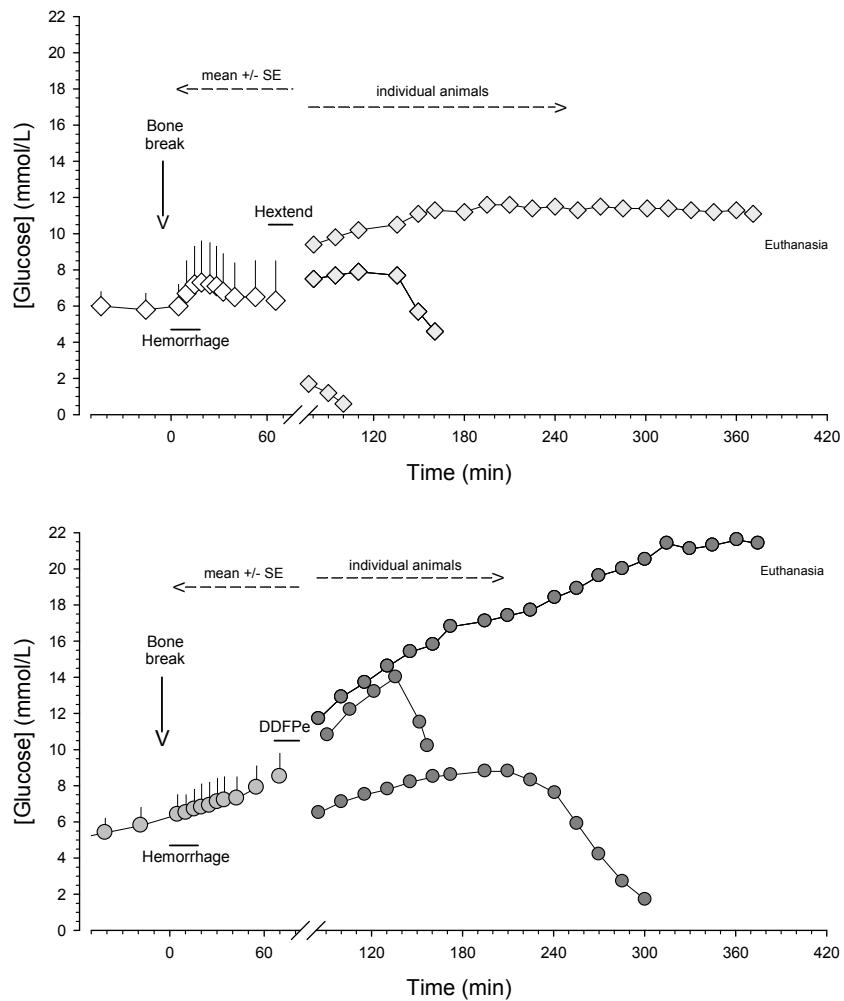


Figure 17: Changes in glucose concentration in arterial blood after bone fracture and hemorrhage are presented in two groups of pentobarbital anesthetized pigs in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For additional details see Figure 11.

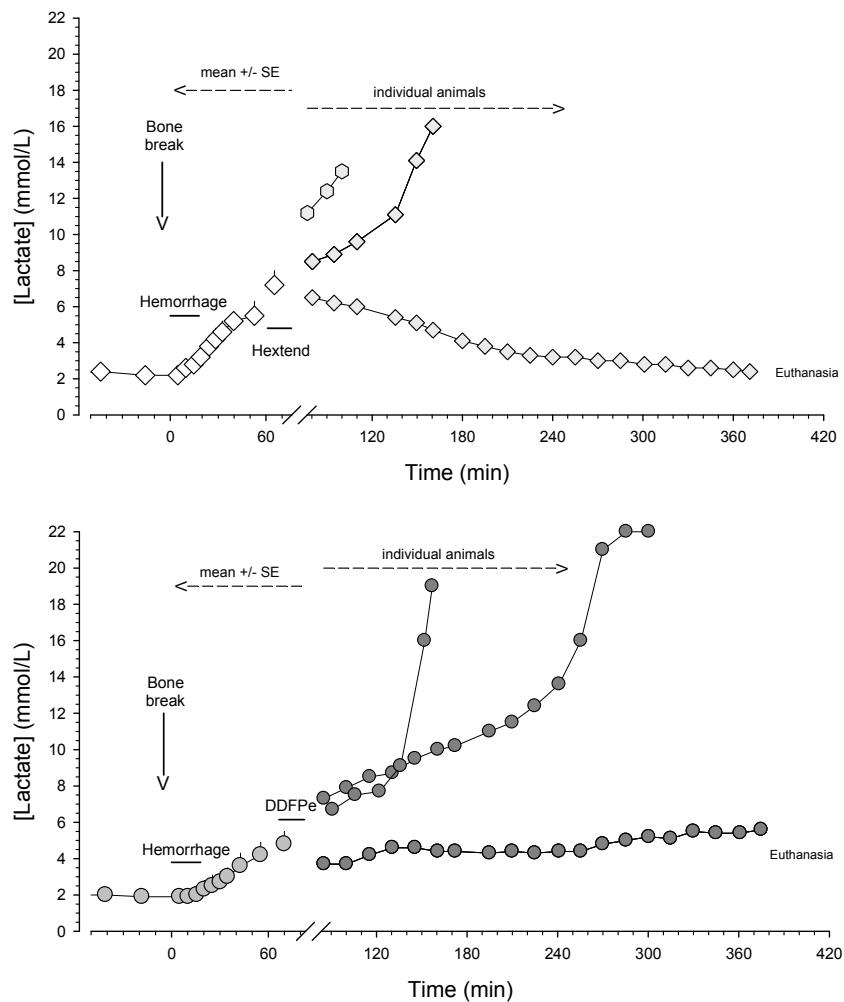


Figure 18: Changes in lactate concentration in arterial blood after bone fracture and hemorrhage are presented in two groups of pentobarbital anesthetized pigs in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For additional details see Figure 11.

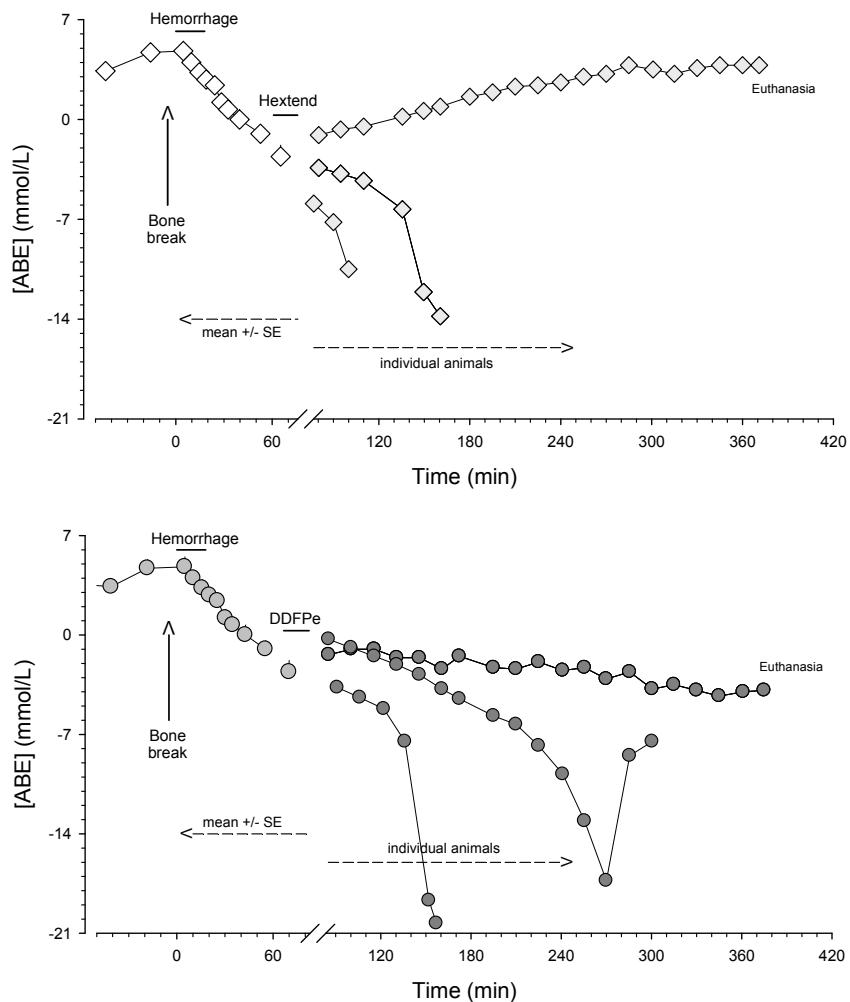


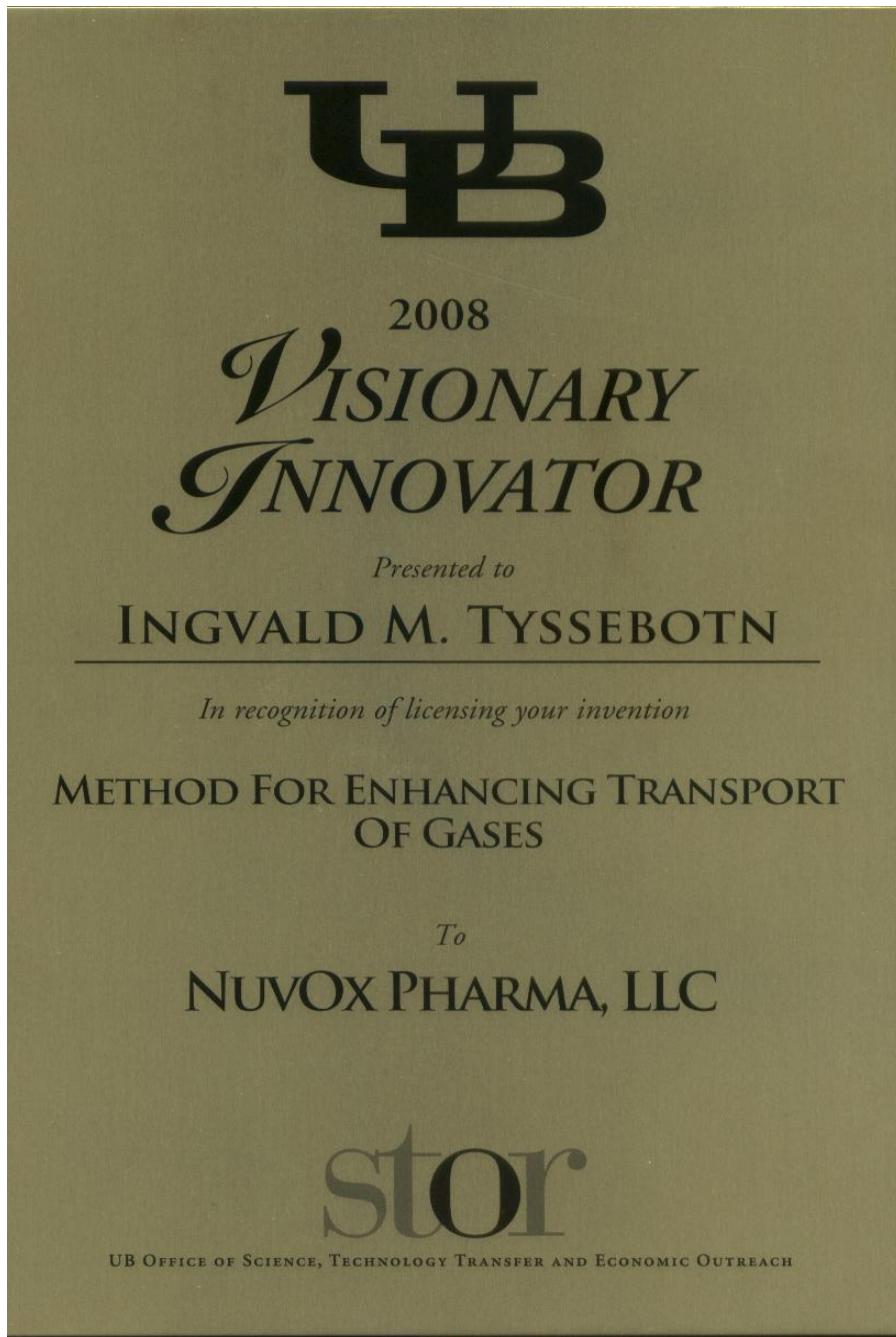
Figure 19: Effects of bone fracture and hemorrhage on actual base excess (ABE) are displayed in two groups of pentobarbital anesthetized pigs. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For further details see Figure 11.

2 – PROOF OF LICENSING

The following US patents were licensed from UB to NuvOx Pharma, LLC in January 2008:

Van Liew, H., Burkard, M.E., Lundgren, C.E .G., Ty ssebotn, I.M. Method for enhancing transport of gases to tissues. US Patent # 5,869,538 (Feb. 9, 1999).

Van Liew, H., Burkard, M.E., Lundgren, C.E .G., Ty ssebotn, I.M. Method for enhancing transport of gases to tissues US Patent # 6,127,428 (2000).



3 - CURRICULUM VITAE (for Principal Investigator)

Ingvald Mikal Tyssebotn

CITIZENSHIP:

Norway

VISA STATUS:

Permanent Resident

ACADEMIC HISTORY:

- 1968: Medical School (Cand Med = M.D.), University of Bergen, Norway
1/5/70: Licensed Physician (Norway, Sweden, Denmark)
1/1/71: Licensed Diving Physician from Norwegian Health Directorate (licensed in Norway and Great Britain for Examination and treatment of North Sea Divers)
12/17/80: Ph.D. in Physiology. Thesis title: Intra-renal Blood Flow in Circulatory Shock and during Infusion of Vasoactive Agents

FURTHER EDUCATION:

- 1963-1965: Basic mandatory military training (The Royal Norwegian Navy – 4 months)
1969: Critical Medical Care, Central Hospital in Sør-Trøndelag, Trondheim, Norway
1969: Adrenals and Its Illness, Aker Hospital, Oslo, Norway
1970: Navy Divers Education, The Royal Norwegian Navy.
1970: Certified instructor for Theoretical and Practical Diver Education, The Royal Norwegian Navy
1970: Officers training (Lieutenant) for Medical Duty in the Royal Norwegian Navy, Sjøkrigsskolen, Bergen, Norway
1971: Experimental Renal Physiology, University of Oslo
1971-1972: Diving Medicine Courses in: Decompression Treatment, Hyperbaric Oxygen Treatment for Diving Accidents and Clinical Illness, The Royal Norwegian Navy and Haukeland Hospital, Bergen, Norway
1972: Pedagogic Course and Lecture Training, University of Bergen, Norway
1972: Clinical Renal Physiology, University of Oslo, Norway
1973: Measurements with Radioactive Isotopes and Radiation protection, The Netherlands-Norwegian School for Isotopic Work, Kjeller, Norway
1974: Radioimmunoassays and Connected Methods, University of Oslo, Norway
1978: Light- Microscopy, EM- and SEM- Microscopy, University of Bergen, Norway.
1979: Clinical Respiratory Physiology, University of Bergen, Norway
1981-1996: Several courses in Diving Medicine, Hyperbaric Oxygen Treatment, and Evaluation of Divers Fitness (Professional and Amateur), approved by the Undersea and Hyperbaric Medical Society and European Undersea Biomedical Society
1994: Hyperbaric Oxygen Treatment, Dept of Anesthesiology, Karolinska Sjukhuset, Stockholm, Sweden
1998: Responsible Care and Use of Laboratory Animals Certification Program, modules 1-4, Institutional Animal Care & Use Committee, Laboratory Animal Facility SUNY at Buffalo.

STUDY TOURS including invited lectureships:

- 1968: Several Universities in Germany (Erlangen, Goettingen, Kiel and Wuerzburg)
1976: Research Fellow at the Queen Elisabeth Hospital, University of Birmingham, Great Britain (6 months), with short Visits to the Universities of Cambridge, Southampton, and London, Lecturing all Places.

- 1978: Defense and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada and The Center for Research and Education in Special Environments, State University of New York at Buffalo, USA
- 1978: Aviation and Naval Medicine Laboratory, Lund University, Sweden
- 1982, 1986, 1987, 1988, 1989, 1992, 1994: Karolinska Institutet, University of Stockholm, Sweden
- 1988: Center for Research & Education in Special Environments (CRESE), State University of New York at Buffalo
- 1988 Schools of Medicine, University of Pennsylvania, Philadelphia, PA

TEACHING:

- 1965-1968: Teaching microscopical and macroscopical anatomy to students of Medical and Dental School, University of Bergen. Supervising research in anatomy. Establishing the medical library for macro and microscopic anatomical preparations used for demonstration, teaching, and examination purposes at Department of Anatomy, University of Bergen
- 1968-1969: Medical diseases, nurses education, Orkdal Sanitetsforenings Hospital, Orkanger, Norway
- 1970-1973: Lecturing Diving Medicine and Diving Physics, Royal Norwegian Navy Diving Courses (including training for Physicians working in the Navy) and Military Diving Units, Fire-Fighters, Oslo, Norway. Lecturing and supervising training of Health Professionals, Royal Norwegian Navy
- 1970-1974: Lecturing physiology, lung diseases and cardiovascular problems, School for Nurses, Betanien sykepleie-skole, Bergen, Norway
- 1971-1998: Lectured at the Department of Physiology, University of Bergen on the following topics:

Renal Physiology: lectures and supervising courses for medical, dental, and science students. Revision of all laboratory journals/manuals

Barophysiology: low and high pressure physiology for medical and science students

Respiratory Physiology: lectures and training courses for medical, science and dental students. Revised all laboratory journals/manuals

Acid-Base Chemistry: lectures and training course for medical, science and dentist students. Revised all experimental journals/manuals

Fluid and Electrolyte Physiology: lectures and training course for medical, science and dental students. Revised laboratory journals/manuals

Basal Metabolism: lectures and training course for medical, science and dental students. Revised the laboratory journals/manuals

Exercise Physiology: lectures and training courses for medical, science and dental students. Revised the experimental journals/manuals

Hemostasis and the Physiology of Erythrocytes: lectures and training courses for medical, science and dental students. Specialty courses for dentists. Revised all journals/manuals

Supervision of master and doctoral students in renal physiology, cardiovascular, hypo- and hyperbaric physiology

Lectured respiratory physiology for physicians with specialty in "Clinical Physiology"

1985-1997: Lectured hyperbaric physiology and diving medicine: nitrogen narcosis, oxygen toxicity, high pressure nervous syndrome, work tolerance at pressure, hyperbaric oxygen treatment for decompression sickness and clinical use at the Norwegian Governmental Diver Training School, Bergen, Norway

1975-1997: Responsible for testing of medical fitness and certification of scientific divers working at the University of Bergen

- 1988: Organized specialty courses in hyperbaric oxygen treatment and diving medicine for Norwegian surgeons at The Central Hospital in Aust-Agder, Kristiansand, Norway
- 1980-1998: Supervisor for use of hyperbaric oxygen for several hospitals in Norway
- 1978-present: Supervised science students studying for the Masters Degree, and Science students and M.D.'s studying for Ph.D Degree. Supervised a total of 16 Masters Degrees and 7 Ph.D. Degrees
- 1985-1994: Supervisor and consultant for research fellows in the Deep Diving Program, the Norwegian Council for Science and the Humanities. Topics: circulation, respiration, oxygen toxicity, gas density.
- 1992: Head of Professor Evaluation Committee, University of Bergen.
- 1992: Organized specialty courses in Hyperbaric Oxygen Treatments for Norwegian Surgeons at Haukeland Hospital, Bergen, Norway.
- 1997-present: Organized PGY50 3 entitled "Physiological measurements in small animals" for graduate students within the School of Medicine & Biomedical Sciences, SUNY at Buffalo.
- 2001-present: Respiratory Physiology, PGY 300, University at Buffalo.
Respiratory Diseases, PGY 412, University at Buffalo.
Renal Diseases, PGY 412, University at Buffalo.

PHD EVALUATIONS:

- 1983: Outside reader Ragnar Vaernes, cand. Psychol. for PhD
- 1999: Outside reader for Andreas Østlund, MD, for PhD thesis, Karolinska Institutet, Stockholm, Sweden.
- 2000: Outside reader for Andreas Fahlman, MSc, for PhD thesis, Carleton University, Ottawa, Canada.

PROFESSIONAL EXPERIENCE:

- 1965-1968: Research Fellow, Department of Anatomy, University of Bergen
- 7/1/68-6/30/69: Intern at Orkdal Sanitetsforenings Hospital, Orkanger, Norway
- 7/1/68-12/31/69: Physician in Sel kommune, Norway
- 1/1/70-2/9/71: The Royal Norwegian Navy, Responsible for training in practical diving and diving medicine at the Navy Diving School, Haakonsvern, Norway
- 2/10/71-1992: Associate Professor, Department of Physiology, University of Bergen, Bergen, Norway
- 7/15/91-8/15/92: Visiting Research Professor, State University of NY at Buffalo, Center for Research & Education in Special Environments (CRESE)
- 1/1/93-8/1/97: Professor of Physiology, Department of Physiology, University of Bergen, Bergen, Norway
- 1/2/94-8/1/97: Consultant, Hyperbaric Medicine, Royal Norwegian Navy
- 1/2/94-8/1/97: Professor of Hyperbaric Medical Research, University of Bergen, Norway
- 1/2/94-8/1/97: Consultant in Hyperbaric Medicine, Haukeland University Hospital, Bergen, Norway
- 8/1/95-8/1/97: Visiting Research Professor, State University of NY at Buffalo, Center for Research & Education in Special Environments (CRESE)
- 8/97-7/31/01: Senior Bertha H. & Henry C. Buswell Fellowship awarded through State University of NY at Buffalo, School of Medicine and Biomedical Sciences
- 8/1/01-present: Research Professor of Physiology and Biophysics, State University of New York at Buffalo.

AWARDS:

- 1972-1995: Yearly grants from The Norwegian Council for Research in the Sciences and the Humanities

3/14/2000: First Place (with co-inventors) Niagara Frontier Inventor of the Year Award in Science. Nomination for this award was made by the University of Buffalo Technology Transfer and Licensing Office and sponsored by the Niagara Frontier Intellectual Property Law Association.

PATENTS:

1. Van Liew, H., Burkard, M.E., Lundgren, C.E.G., Tyssebotn, I.M. Method for enhancing transport of gases to tissues. US Patent # 5,869,538 (Feb. 9, 1999).
2. Van Liew, H., Burkard, M.E., Lundgren, C.E.G., Tyssebotn, I.M. US Patent # 6,127,428 (2000).

PUBLICATIONS:

Articles in peer reviewed journals:

1. Aukland, K., Kirkeboe, A., Løyning, E. and Tyssebotn, I. Effect of hemorrhagic hypotension on the distribution of renal cortical blood flow in anesthetized dog. *Acta Physiol Scand*, 87, 514-525, 1973.
2. Clausen, G. and Tyssebotn, I. Intrarenal distribution of glomerular filtration in conscious rats during isotonic saline infusion. *Acta Physiol Scand*, 89, 289-295, 1973.
3. Clausen, G. and Tyssebotn, I. Single-nephron filtration during hemorrhagic hypotension in the conscious rats. *Acta Physiol Scand*, 92, 364-373, 1974.
4. Kirkeboe, A. and Tyssebotn, I. Distribution of renal cortical blood flow during hemorrhagic hypotension in conscious dogs. *Acta Physiol Scand*, 91, 22-31, 1974.
5. Tyssebotn, I. and Kirkeboe, A. Effect of vasoactive agents on the distribution of renal cortical blood flow in dogs. *Acta Physiol Scand*, 95, 318-328, 1975.
6. Kirkeboe, A. and Tyssebotn, I. Effect of dehydration on renal blood flow in dog. *Acta Physiol Scand*, 101, 257-263, 1977.
7. Tyssebotn, I. and Kirkeboe, A. The effect of indomethacin on renal blood flow distribution during hemorrhagic hypotension in dog. *Acta Physiol Scand*, 101, 15-21, 1977.
8. Koller, M.E., Romslo, I., Finne, P.H., Brochmeier, F. and Tyssebotn, I. The diagnoses of iron deficiency by erythrocyte protoporphyrin and serum ferritin analyses. *Acta Paediatr Scand*, 67, 361-367, 1978.
9. Tyssebotn, I. and Kirkeboe, A. Renal cortical blood flow distribution measured by hydrogen clearance during dopamine and acetylcholine infusion. Effect of electrode thickness and position in cortex. *Acta Physiol Scand*, 106, 385-393, 1979.
10. Clausen, G., Kirkeboe, A., Tyssebotn, I., Øfjord, E.S. and Aukland, K. Erroneous estimates of intrarenal blood flow distribution in the dog with radiolabeled microspheres. *Acta Physiol Scand*, 107, 385-387, 1979.
11. Clausen, G., Hope, A., Kirkeboe, A., Tyssebotn, I. and Aukland, K. Distribution of blood flow in the dog kidney. I. Saturation rates for inert diffusible tracers, ^{125}I -iodoantipyrine and tritiated water, versus uptake of microspheres under control conditions. *Acta Physiol Scand*, 107, 69-81, 1979.
12. Clausen, G., Hope, A., Kirkeboe, A., Tyssebotn, I. and Aukland, K. Distribution of blood flow in the dog kidney. II. Saturation rates of inert diffusible tracers versus uptake of $15\text{ }\mu\text{m}$ microspheres during vasodilation and vasoconstriction. *Acta Physiol Scand*, 110, 249-258, 1980.
13. Kirkeboe, A. and Tyssebotn, I. Renal blood flow distribution during *E.coli* endotoxin shock in dog. *Acta Physiol Scand*, 108, 367-372, 1980.
14. Tyssebotn, I. and Kirkeboe, A. Patchy, intermittent ischemia in renal cortex during tourniquet shock in dog. *Acta Physiol Scand*, 109, 253-260, 1980.

15. Onarheim, J. and Tyssebotn, I. Effect of high ambient pressure and oxygen tension on organ blood flow in the anesthetized rats. *Undersea Biomed Res*, 7, 47-60, 1980.
16. Clausen, G., Kirkeboe, A., Tyssebotn, I. and Aukland, K. Distribution of blood flow in the dog kidney. III. Local uptake of 10 µm and 15 µm microspheres during renal vasodilation and constriction. *Acta Physiol Scand*, 113, 481-485, 1981.
17. Clausen, G., Kirkeboe, A., Tyssebotn, I. and Aukland, K. Distribution of blood flow in the dog kidney. IV. Reversed net inward postglomerular capillary flow in the cortex after blocking interlobular arteries by 50 µm microspheres. *Acta Physiol Scand*, 113, 481-485, 1981.
18. Johannessen, W.M., Tyssebotn, I. and Aarbakke, J. Antipyrine and acetaminophen kinetics in the rat. Comparison of data base and on blood samples from the cut tail and a cannulated femoral artery. *J Pharma Sci*, 71, 1352-1356, 1982.
19. Hope, A., Tyssebotn, I. and Clause n, G. The effect of hemorrhagic hypotension on total and local renal blood flow in the rat. *Renal Physiol*, 6, 43-52, 1983.
20. Hope, A. and Tyssebotn, I. The effect of water deprivation on local renal blood flow and filtration in the laboratory rat. *Circ Shock*, 11, 175-186, 1983.
21. Sundberg, H, Værnes, R. and Tyssebotn, I. The role of hyperoxia in the possible long term neurological effects of diving. Minipaper, *Acta Neurolog Scand*, 169, 140-141, 1984.
22. Kirkeboe, A, Haugan, A and Tyssebotn, I. Blood flow heterogeneity in the renal cortex during burn shock in dogs. *Acta Physiol Scand*, 123, 205-213, 1985.
23. Aanderud, L., Onarheim, J. and Tyssebotn, I. The effect of 71 ATA He-O₂ on organ blood flow in the rat. *J Appl Physiol*, 1369-1375, 59, (5), 1985.
24. Hordnes, C. and Tyssebotn, I. Effect of high ambient pressure and oxygen tension on organ blood flow in conscious trained rats. *Undersea Biomed Res*, 12, 115-128, 1985.
25. Risberg, J. and Tyssebotn, I. Hyperbaric exposure to 5 ATA in a He-N₂-O₂ atmosphere affects the cardiac function and organ blood flow in awake rats. *Undersea Biomed Res*, 13, (1),77-90, 1986.
26. Risberg, J., Hordnes, C. and Tyssebotn, I. The effect of β₁-adrenoceptor blockade on cardiac output and organ blood flow in conscious trained rats. *Scandinavian J Clin Lab Invest*, 47, 521-527, 1987.
27. Ask, J.A. and Tyssebotn, I. Effect of compression to 5, 10, and 30 Bar on the contractile activity of isolated atrial preparations from the rat heart. *Underwater Physiology IX*, Bethesda, MD, 465-469, 1987.
28. Bergø, G.W., Ask, J.A. and Tyssebotn, I. Breathing gas density influences the myocardial contractility. Minipaper, *Underwater Physiol*, IX, Bethesda, MD, 471-476, 1987.
29. Ask, J.A. and Tyssebotn, I. Inotropic and chronotropic effects of noradrenaline via cardiac beta-adrenoceptors during hyperbaric exposure. *Undersea Biomed Res*, 1987.
30. Ask, J.A. and Tyssebotn, I. Positive inotropic effect on the rat atrial myocardium compressed to 5, 10, and 30 bar. *Acta Physiol Scand*, 134, 277-283, 1988.
31. Bergø, G.W., Risberg, J. and Tyssebotn, I. Effect of 5 bar oxygen on cardiac output and organ blood flow in conscious rats. *Undersea Biomed Res*, 15, 457-470, 1988.
32. Furset, K., Aanderud, L., Segadal, K. and Tyssebotn, I. Transcutaneous measurements of P_{CO₂} high ambient pressure (41 bar). *Undersea Biomed Res*, 14, (4), 51-62, 1988.
33. Stuhr, L.E.B., Ask, J.A. and Tyssebotn, I. Increased cardiac contractility in rats exposed to 5 bar. *Acta Physiol Scand*, 136, 167-176, 1989.
34. Furset, K, Aanderud, L. and Tyssebotn, I. Respiratory depression by analgesics at 41 bar. *Undersea Biomed Res*, 16, 219-226, 1989.
35. Mæhle, B.O., Giertsen, J.C.H. and Tyssebotn, I. Hypertrophy of the left cardiac ventricle in professional divers. *J Hyperbaric Medicine*, 4, (4), 189-195, 1989.
36. Risberg, J., Bergø, G.W., Hordnes, C. and Tyssebotn,I. Distribution of cardiac output in awake rats during exposure to 5 bar. *Undersea Biomed. Res.*, 17(6): 503-514, 1990.

37. Stuhr, L.E.B., Ask, J.A. and Tyssebotn, I. Cardiovascular changes in anesthetized rats during exposure to 30 bar. *Undersea Biomed Res*, 17, (5), 383-393, 1990.
38. Ask, J.A. and Tyssebotn, I. Positive inotropic effect on the human atrial myocardium exposed to 30 bar. *Undersea Biomed Res*, 18, (2), 138-143, 1991.
39. Bergø, G.W. and Tyssebotn, I. Respiratory frequency and distribution of cardiac output in rats breathing gas with different densities. *Scand. J. Clin. Lab. Invest.* 51:59-66, 1991.
40. Stuhr, L.E.B., Ask, J.A. and Tyssebotn, I. Inotropic and chronotropic responses to isoprenaline in rats exposed to 30 bar. *Aviat Space Environ Med*, 62, 41-45, 1991.
41. Bergø, G.W. and Tyssebotn, I. Increased breathing gas density enhances cardiac work load. *Scand. J. Clin. Lab. Invest.* 52:151-158, 1992.
42. Bergø, G.W. and Tyssebotn, I. Cerebral blood flow distribution during exposure to 5 bar oxygen in conscious rats. *Undersea Biomed. Res.* 19:339-354, 1992.
43. Risberg, J. and Tyssebotn, I. Organ blood flow and cardiac contractility in anaesthetized cats at 5 bar (300 kPa) ambient pressure. *Europ J Appl Phys*, 64, 389-394, 1992.
44. Stuhr, L.E.B., Bergoe, G.W., Skei, S., Mæhle, B.O. and Tyssebotn, I. Repeated normoxic hyperbaric exposures induce hemodynamic and myocardial changes in rats. *Euro J Appl Physiol* 66(3): 224-234, 1993.
45. Bergø, G.W., Engelsen, B. and Tyssebotn, I. Unilateral frontal decortication changes cerebral blood flow distribution during hyperbaric oxygen exposure in rats. *Aviat Space Environ Med* 64: 1023-1031, 1993.
46. Bergø, G.W. and Tyssebotn, I. Cerebral blood flow distribution and systemic hemodynamics changes after repeated exposures to 5 bar oxygen. *Euro J Appl Physiol*, 69:1-9, 1994.
47. Stuhr, L.E.B., Bergø, G.W. and Tyssebotn, I. Systemic hemodynamics during hyperbaric oxygen exposure in rats. *Aviat Space Environ Med*, 65:531-538, 1994.
48. Bergø, G.W. and Tyssebotn, I. Repeated exposures to 5 bar normoxic He-N₂ changes the cerebral blood flow distribution in rats. *J Appl Physiol*, 78 (6):2109-2114, 1995.
49. Risberg, J., Hordnes, C., Stuhr, L.E.B. and Tyssebotn, I. Effects of β_1 -adrenoceptor blockade in rats at 5 bar ambient pressure. *Undersea Hyperbaric Med* 21, 371-385, 1994.
50. Bergø, G.W. and Tyssebotn, I. Cerebral blood flow distribution and systemic hemodynamics during 3 bar oxygen exposure in rats given 2 kPa (15 mmHg) CO₂ in the breathing gas. *J Appl Physiol*, 78 (6): 2100-2109, 1995.
51. Risberg, J., Skei, S. and Tyssebotn, I. Effect of gas density and ambient pressure on myocardial contractility in the rat. *Aviat Space Envir Med*, 66: 1159-1168, 1995.
52. Bergø, G.W. and Tyssebotn, I. Effect of 101 and 150 kPa oxygen on the cerebral circulation and oxygen supply in conscious rats. *Euro J Appl Physiol*, 71 (6): 475-484, 1995.
53. Jiang, J. and Tyssebotn, I. A model for acute monoxide poisoning in conscious rats. *Undersea Hyperbaric Med. Undersea and Hyperbaric Medicine* 23(2):99-106, 1996.
54. Krossnes, BK, Mella, O. and Tyssebotn, I. Effect of sodium nitroprusside-induced hypotension on the blood flow in subcutaneous and intramuscular BT4AN tumors and normal tissues in rats. *Int J Radiation Oncology Biol Phys* 36(2):393-401, 1996.
55. Jiang, J. and Tyssebotn, I. Measurement of cerebrospinal fluid pressure in conscious rats. *Undersea & Hyperbaric Medicine*. 24:39-43, 1997.
56. Jiang, J. and Tyssebotn, I. Normobaric and hyperbaric oxygen treatment of acute carbon monoxide poisoning in rats. *Undersea Hyperbaric Med*, 24(2):107-116, 1997.
57. Jiang, J. and Tyssebotn, I. Cerebrospinal fluid pressure changes after acute carbon monoxide poisoning and therapeutic effects of normobaric and hyperbaric oxygen in conscious rats. *Undersea Hyper Med* 24(4):245-254, 1997.
58. Bergø, G.W. and Tyssebotn, I. Cardiovascular effects of hyperbaric oxygen with and without addition of carbon dioxide. *Eur J Appl Physiol*, 80:264-275, 1999.

59. Stuhr, L.E.B., Risberg, J., Bergø, G.W., and Tyssebotn, I. Cardiovascular effects of verapamil and quinidine at normal and elevated ambient pressure. *Aviat Space Environ Med* 72:373-379, 2001.
60. Lundgren CEG, Bergoe GW, Tyssebotn I. Hemorrhagic shock in air breathing pigs treated with bubble-forming intravenous dodecafluoropentane emulsion. *Artif Blood* 11(1); F-1-4, 2003.
61. Lundgren CEG, Bergoe GW, Tyssebotn I. The theory and application of intravascular microbubbles as an ultra-effective means of transporting oxygen and other gases. *Undersea & Hyper Med* 31(1): 105-6,2004
62. Lundgren C, Bergoe G, Olszowka A, Tyssebotn I. Tissue nitrogen elimination in oxygen-breathing pigs is enhanced by fluorocarbon-derived intravascular microbubbles. *Undersea and Hyperbaric Medicine* 32(4): 215-226, 2005
63. Lundgren CE, Bergoe GW, Tyssebotn IM. Intravascular fluorocarbon-stabilized microbubbles protect against fatal anemia in rats. *Artificial Cells, Blood Substitutes, & Immobilization Biotechnology*. 34(5): 473-86, 2006.

Book chapters:

1. Kirkeboe, A. and Tyssebotn, I. Intrarenal blood flow in circulatory shock and during infusion of vasoactive agents. Ph.D. thesis (Joint chapter of Ph.D. Thesis), pp. 1-52, 1980.
2. Kirkeboe, A. and Tyssebotn, I. Measurement of regional blood flow in kidney. In: *Acute Renal Failure* (Seybold, D and Gesler, U eds.), Karger, Basel, 32-40, 1982.
3. Tyssebotn, I. and Kirkeboe, A. Patchy, renal ischemia during hypovolemic shock in dog. In: *Acute Renal Failure* (Seybold, D and Gesler, U eds.), Karger, Basel, 23-32, 1982.
4. Tyssebotn, I. Svømmedykking ved Universitetet i Bergen. Revision, 1984.
5. Risberg, J., Hordnes, C. and Tyssebotn, I. The effect of β_1 -blockade on the distribution on cardiac output at normal and increased ambient pressure in conscious rats. *Underwater physiology VIII* (Bachrach AJ and Matzen, MM eds.), Undersea Med Soc, USA, 309-314, 1984.
6. Arntzen, A.J. and Eidsvik, S. Nye norske dykketabeller, Revision, Bergen 1991.

Proceedings from International Conferences and Meetings:

1. Ask, J.A. and Tyssebotn, I. Positive inotropic effects of rat atrial myocardium compressed to 5, 10, and 30 Bar. Minipaper, Proceeding EUBS 86 on diving and hyperbaric medicine, pp. 65-69, 1986.
2. Stuhr, L.E.B., Ask, J. A. and Tyssebotn, I. Increased inotropy of the heart in normoxic hyperbaric atmosphere. Minipaper, Proceeding EUBS 85 on diving and hyperbaric medicine, Göteborg, Sweden, pp. 133-138, 1985.
3. Bergø, G.W. and Tyssebotn, I. Repeated exposures to 5 Bar oxygen change cerebral blood flow distribution in awake, trained rats. Minipaper, Proceedings EUBS 86 on diving and hyperbaric medicine, pp. 17-23, 1986.
4. Bergø, G.W. and Tyssebotn, I. How will hyperbaric oxygen influence the pump work of the rat heart? Minipaper, Proceedings EU BS 87 on diving and hyperbaric medicine, Palermo, Italy, pp. 154-160, 1987.
5. Bergø, G.W., Engelsen, B. and Tyssebotn, I. Regional cerebral blood flow (rCBF) distribution after unilateral cortical lesions during hyperbaric (HBO) in rats. Minipaper, Proceedings EUBS 87 on diving and hyperbaric medicine, pp. 161-166, 1987.
6. Ask, J.A., Hansen, A. and Tyssebotn, I. Inotropic effect of noradrenaline via cardiac beta-adrenoceptors at 30 and 60 bar. Minipaper, Proceedings EUBS 87 on diving and hyperbaric medicine, Palermo, Italy, pp. 258-263, 1987.

7. Stuhr, L.E.B., Ask, J. A. and Tyssebotn, I. Effect of exposure to 3-0 bar on the cardiac contractility of anesthetized rats. Minipaper, Proceedings E UBS 87 on diving and hyperbaric medicine, Palermo, Italy, pp. 254-257, 1987.
8. Tyssebotn, I. Physiological effects of breathing increased partial pressure of oxygen. Oljedirektoratets konferanse i Stavanger, 3.-4. Februar, 1988.
9. Risberg, J, Stuhr, L.E.B. and Tyssebotn, I. Increased cardiac contractility in the anesthetized rat at 5 bar ambient pressure. Proceedings XVIth Annual Meeting EUBS, Amsterdam, Netherland, pp. 189-195, 1990.
10. Bergø, G.W and Tyssebotn, I. Circulatory changes after repeated exposures to 4 bar oxygen in awake rats. Proceedings of the 10th International Congress of Hyperbaric Medicine, Amsterdam, Netherland, pp. 52-59, 1990.
11. Risberg, J., Stuhr, L.E.B. and Tyssebotn, I. Increased cardiac contractility in the anesthetized rat at 5 bar ambient pressure. Proceedings XVIth Annual Meeting EUBS, Amsterdam, Netherland, pp. 189-195, 1990.
12. Risberg, J., Stuhr, L.E.B. and Tyssebotn, I. Increased cardiac contractility in the anesthetized rat at 5 bar ambient pressure. Proceedings of the 10th International Congress of Hyperbaric Medicine, Amsterdam, Netherland, pp. 52-59, 1990.
13. Stuhr, L.E.B, Bergø, G.W., Risberg, J. and Tyssebotn, I. Effekten av ulike puste gasser på hjertets pumpetrykk og kontraktilitet. Vitenskapelig FUDT-seminar, Bergen, Norway, pp. 49-52, 1990.
14. Bergø, G.W . and Tyssebotn, I. Blodstroemsfordelingen i kroppen og hjertets arbeid ved varierende oksygeninnhold i pustegassen. Vitenskapelig FUDT- seminar, Bergen, Norway, pp. 20-23, 1990.
15. Tyssebotn, I, Stuhr, L.E.B. and Bergø, G.W. Relative influence of different factors on the heart during diving. Proceedings of the 10th International Congress of Hyperbaric Medicine, Amsterdam, Netherland, pp. 52-59, 1990.
16. Bergø, G.W. and Tyssebotn, I. Circulatory changes after repeated exposures to 4 bar oxygen in awake rats. Proceedings of the Tenth International Congress on Hyperbaric Medicine, Ed: DJ.Bakker, Best Publishing Company, Flagstaff, AZ., ISBN 0-941332-24-1, pp. 52-59, 1992.
17. Hope, A., Bergø, G.W, and Tyssebotn, I. Quantification of central venous gas bubbles after exposure to 5 bar in conscious rats. Proceedings XXth Annual Meeting EUBS, Istanbul, Turkey, ISBN 975-7958-00-X, pp. 106-108, 1994.
18. Jiang, J and Tyssebotn, I. Acute effects of reduced ambient pressure on intracranial pressure in anesthetized rats. Proceedings XXth Annual Meeting EUBS, Istanbul, Turkey, ISBN 975-7958-00-X, pp. 402-406, 1994.
19. Jiang, J and Tyssebotn, I. Acute effects of elevated ambient pressure on intracranial pressure in anesthetized rats. Proceedings XXth Annual Meeting EUBS, Istanbul, Turkey, ISBN 975-7958-00-X, pp. 407-412, 1994.
20. Lundgren C., Bergø G. and Tyssebotn I. Intravascular Microbubbles: an Ultra-Effective Means of Transporting Oxygen, in Artificial Oxygen Carrier: Its Frontline. Keio University International Symposia for Life Sciences and Medicine, Vol. 2. Chapter xx Springer-Verlag Tokyo, 2004

Abstracts

1. Aukland, K., A. Kirkeboe, E. Loeyning and I. Tyssebotn. Distribution of renal cortical blood flow in hemorrhagic hypotension. In: Abstr Vth Int Congr Nephrology Mexico 1972, 70.
2. Aukland, K., A. Kirkeboe, E. Loeyning and I. Tyssebotn. Distribution of renal cortical blood flow during hemorrhagic hypotension in dogs. Acta Physiol Scand 84: 11A- 12A. 1972.

3. Clausen, G. and I. Tyssebotn. Intrarenal distribution of glomerular filtration in conscious rats during isotonic saline infusion and hemorrhagic hypotension. *Acta Physiol Scand* 87:34A-35A, 1973.
4. Tyssebotn, I. and A. Kirkeboe. Distribution of renal cortical blood flow during infusion of vasoactive agents in dog. *Microvasc Res* 6:256, 1973.
5. Aukland, K., A. Kirkeboe and I. Tyssebotn. Intarenal blood flow in dogs in dehydration and shock. *J Physiology* 245:99-100, 1974.
6. Kirkeboe, A. and I. Tyssebotn. Renal cortical blood flow during dehydration in dogs. *Acta Physiol Scand* 91:5A-6A, 1974.
7. Kirkeboe, A. and I. Tyssebotn. Renal cortical blood flow pattern in dehydrated dogs. *Europ Colloq Physiol Inserm* 30:172, 1974.
8. Clausen, G., A. Hope, A. Kirkeboe, I. Tyssebotn and K. Aukland. Effect of vasodilation on distribution of microspheres and on zonal blood flow measured with diffusible indicators in the dog kidney. *Proc Internat Union Physiol Sci* 13:141, 1977.
9. Clausen G., A. Hope, A. Kirkeboe, I. Tyssebotn and K. Aukland. Selective vasodilation in deep renal cortex. *Proceedings II. European Colloquium on Renal Physiology* Ungarn p. 19, 1977.
10. Kirkeboe, A. and I. Tyssebotn. Vasoconstrictor effect of indomethacin in the dog kidney. *Proceedings II. European Colloquium on Renal Physiology* Ungarn p. 65, 1977.
11. Clausen, G., A. Hope, A. Kirkeboe, I. Tyssebotn and K. Aukland. Glomerular versus postglomerular capillary blood flow. *Int Congr Nephrol* 7th Montreal p. F-12, 1978.
12. Onarheim, J. and I. Tyssebotn. Effect of high ambient pressure and oxygen tension on organ blood flow in the rat. *Acta Physiol Scand* 1979.
13. Onarheim, J. and I. Tyssebotn. High ambient pressure changes the distribution of cardiac output in anesthetized rats. *EUBS*. 1979.
14. Clausen, G., A. Kirkeboe, I. Tyssebotn, E.S. Oefjord and K. Aukland. Skimming of microspheres causes false estimates of blood flow distribution in the dog kidney. *Acta Physiol Scand* 108:6A, 1980.
15. Tyssebotn, I. and A. Kirkeboe. Heterogeneous blood flow in renal cortex during tourniquet shock. *Acta Physiol Scand* 108:15A, 1980.
16. Onarheim, J., C. Hordnes and I. Tyssebotn. Increased inotropic response caused by high ambient pressure. *Acta Physiol Scand* 114:22A, 1982.
17. Hordnes, C., I. Tyssebotn and J. Onarheim. Effect of high ambient pressure and oxygen tension on organ blood flow in conscious rats. *Acta Physiol Scand* 114:23A, 1982.
18. Kirkeboe, A., I. Tyssebotn and A. Haugan. Heterogen gjennomblødning i nyre bark under forbrenningsjøkk. *Nordisk seminar om nyresirkulasjon* Bergen 1983.
19. Bergø, G., L. Aanderud and I. Tyssebotn. Blood flow in the inner ear at 71 ATA in conscious rats. *Eur Undersea Biomed Soc* 1984.
20. Risberg J., C. Hordnes and I. Tyssebotn. The effect of high ambient pressure on organ blood flow in conscious rats. *Eur Undersea Biomed Proceeding* 1984.
21. Aanderud, L., J. Onarheim and I. Tyssebotn. Does high pressure alter central hemodynamic? *Eur Undersea Biomed Soc* 1984.
22. Bergø, G. W. and I. Tyssebotn. Circulatory changes in hyperbaric oxygen exposed rats. *Proceeding EUBS85*, p. 15, 1985.
23. Bergø, G.W. and I. Tyssebotn. Light and dense breathing gas changes coronary blood flow differently in anesthetized rats. *Proceeding EUBS 85*, Göteborg, Sweden, p. 14, 1985.
24. Bergø, G.W. and I. Tyssebotn. Hyperbaric oxygen changes the blood flow distribution in the brain of awake, trained rats. *Acta Physiol Scand*, 1986.
25. Stuhr, L.B., J.A. Ask and I. Tyssebotn. Increased inotropy of the heart in normoxic hyperbaric atmosphere. *Acta Physiol Scand* 1986.

26. Bergø, G.W. and I. Tyssebotn. Heart performance during hyperbaric oxygen (HBO) exposure in awake, habituated rats. Undersea Biomed Res, 15, suppl, 54, 1988.
27. Furset, K., Aanderud, L. and I. Tyssebotn. Respiratory depression after morphine and fentanyl at high ambient pressure (41 bar). Undersea Biomed Res, 15, suppl, 77-78, 1988.
28. Ask, J.A., Stuhr, L.E.B. and I. Tyssebotn. Cardiac contractility and adrenergic regulation of the heart under hyperbaric conditions. Proceedings, XIXth Nordic Congress of Physiology and Pharmacology, 13-15th June, Oslo, Norway, 23, 1988.
29. Furset, K., L. Aanderud and I. Tyssebotn. Re-spirasjonsdepresjon av morfin og fentanyl ved høyt omgivende trykk, 41 ATA. Norsk Anestesiologisk Forening, Meeting Oct. 1988.
30. Mæhle, B.O., J. Chr. Giertsen and I. Tyssebotn. Does diving cause hypertrophy of the left cardiac ventricle? International Symposium and Underwater-Hyperbaric Medicine and Technology, Athens, Greece, October 1988.
31. Stuhr, L.B., G.W. Bergø, B.O. Mæhle, S. Skei, J.A. Ask and I. Tyssebotn. Hyperbaric exposures of rats lead to hypertrophy of the left ventricular myocardium. 5. International Symposium and Underwater-Hyperbaric Medicine and Technology, Athens, Greece, October 1988.
32. Stuhr, L.B., J.A. Ask and I. Tyssebotn. Effect of hyperbaric oxygen (5 bar) on the cardiac contractility of anesthetized rats. Undersea Biomed Res, 15, suppl, 30, 1988.
33. Stuhr, L.B., J.A. Ask and I. Tyssebotn. In vivo activation of cardiac beta-adrenoceptors by isoprenaline at 30 bar. Undersea Biomed Res, 15: suppl, p. 47, 1988.
34. Stuhr, L.E.B., G.W. Bergø and I. Tyssebotn. Cardiovascular effects of normoxic 5 bar on rats after 40 repeated hyperbaric exposures. Undersea Biomed Res, suppl, 16, 64, 1989.
35. Bergø, G.W. and I. Tyssebotn. Calculated pump work of the rat heart during exposure to oxygen at 1, 3, and 5 bar. Undersea Biomed Res, 16 suppl, p. 97, 1989.
36. Bergø, G.W. and I. Tyssebotn. Organ blood flow in conscious rats exposed to 1, 3 and 5 bar oxygen. Proceeding XVth Annual Meeting EUBS 89, Eilat, Israel, 17-21 September, 1989.
37. Stuhr, L.B., G.W. Bergø, S. Skei, B.O. Mahle, J.A. Ask and I. Tyssebotn. Effects of repeated hyperbaric exposure on the rat heart. Undersea and Hyperbaric Medical Society Annual Scientific Meeting 6-11 June 1989. Undersea Biomed. Res. Suppl. Vol 16, p. 63.
38. Bergø, G.W. and I. Tyssebotn. Regional cerebral blood flow during exposure to 1, 3 and 5 bar oxygen. Undersea Biomed Res, Suppl, 16, 75, 1989.
39. Bergø, G.W., and I. Tyssebotn. Organ blood flow in conscious rats exposed to 1, 3 and 5 bar oxygen. XVth Annual Meeting EUBS 89, Eilat, Israel, 17-21 September 1989.
40. Bergø, G.W. and I. Tyssebotn. Blood flow in the brain and the heart during oxygen exposure. XVth Annual Meeting EUBS 89, Eilat, Israel, 17-21 September, 1989.
41. Bergø, G.W., L.E.B. Stuhr, S. Skei, and I. Tyssebotn. The effect of normoxic hyperbaric exposure on the myocardial blood flow and contractility in conscious rats. XVth Annual Meeting EUBS 89, Eilat, Israel, 17-21 September, 1989.
42. Bergø, G.W. and I. Tyssebotn. Calculated pump work of the rat heart during exposure to oxygen at 1, 3, and 5 bar. Undersea Biomed Res, Suppl, 16, 97, 1989.
43. Stuhr, L.E.B., G.W. Bergø, S. Skei, B.O. Mæhle, J.A. Ask, and I. Tyssebotn. Effects of repeated hyperbaric exposure on the rat heart. Undersea Biomed Res, Suppl, 16, 63, 1989.
44. Bergø, G.W. and I. Tyssebotn. Cerebral blood flow distribution after repeated exposure to 4 bar oxygen in awake rats. Proceedings of the 10th International Congress of Hyperbaric Medicine, Amsterdam, Netherland, 52-59, 1990.
45. Risberg, J., L.B. Stuhr and I. Tyssebotn. Increased cardiac contractility in the atenolol treated rat at 5 bar ambient pressure. UHMS meeting, Amsterdam, August 1990.
46. Ask, J.A. and I. Tyssebotn. Positive inotropic effect on human atrial myocardium exposed to 30 bar. International group on high pressure biology. Toulon, France, August 1990.

47. Tyssebotn, I., L.B. Stuh r and G. Be rgø. Relative influence of different factors on the heart during diving. International Congress on Hyperbaric Medicine, Amsterdam, August 1990.
48. Bergø, G.W. and I. Tyssebotn. Circulatory changes after repeated exposure to 4 bar oxygen in awake rats. International Congress on Hyperbaric Medicine, Amsterdam, August 1990.
49. Tyssebotn, I. Hvorfor t renger vi d ykkfysiologisk forskning på dyr? Hva kan o verføres til menneske fra slire for sørk? Popularvitenskapelig FUDT- seminar, Bergen, Norway, p. 70, 1990.
50. Ask, J.A. and I. Tyssebotn. Positive inotropic effect on human atrial myocardium exposed to 30 bar. International group on high pressure biology. Undersea Biomed Res, 18, (2), 138, 1991.
51. Bergø, G.W. and I. Tyssebotn. Cerebral blood flow distribution and systemic hemodynamics during 3 bar oxygen exposure in rats given 2 kPa (15 mmHg) CO₂ in the breathing gas. Proceeding of the XIXth EUBS Annual Meeting, p.220, August 1993.
52. Bergø, G.W. and I. Tyssebotn. Repeated exposures to 5 bar normoxic He-N₂ changes the cerebral blood flow distribution in rats. Proceeding of the XIXth EUBS Annual Meeting, p.109, August 1993.
53. Bergø, G.W . and I. Tyssebotn. Evaluation of decompression profiles from saturation in conscious rats. Acta Physiol Scand, 1994.
54. Skjolde, S., G.W. Bergø and I. Tyssebotn. Increased breathing resistance affects cardiac performance. Acta Physiol Scand, 1994.
55. Hope, A., G.W. Bergø and I. Tyssebotn. A new method for detecting central venous gas bubbles during decompression in rats. Acta Physiol Scand, 1994.
56. Eikemo, S.H. and I. Tyssebotn. The effect of cold breathing gas on cardiac performance in rats. Acta Physiol Scand, 1994.
57. Jiang, J. and I. Tyssebotn. Effects of high and low ambient pressure on the intracranial pressure in rats. Acta Physiol Scand, 1994.
58. Jiang, J. and I. Tyssebotn. Effect of normobaric and hyperbaric O₂ on acute carbon monoxide poisoning in rats. UHMS meeting 1996.
59. Bergø, G.W. and I. Tyssebotn. Organ blood flow and cardiac performance in rats during and after repeated exposure to 500 kPa normoxic He-N₂. UHMS meeting, 1996.
60. Tyssebotn, I., Lundgren C., Bergø G, Van Liew H, Goldinger J. Stabilized microbubbles as a blood substitute and hemoglobin complement. Presented at the 1999 UHMS Annual Meeting, held at the Westin Copley Place, Boston, MA, June 26-30, 1999. Undersea & Hyperbaric Medicine 26(suppl):96, 1999.
61. Tyssebotn, I., Bergø G, Goldinger J, Lundgren C. Dodecafluoropentane (DDFP) stabilized microbubbles support life without blood in awake rats. Presented at the 1999 UHMS Annual Meeting, held at the Westin Copley Place, Boston, MA, June 26-30, 1999. Undersea & Hyperbaric Medicine 26(suppl):97, 1999.
62. Lundgren C, Bergø G, Olszowka A, Logue G, Tyssebotn I. Intravascular microbubbles used for successful treatment of right-to-left circulatory shunts in the lung. Presented at the 1999 Annual UHMS Scientific Meeting held at the Westin Copley Place, Boston, MA, June 26-29, 1999. Undersea & Hyperbaric Medicine 26(suppl):98, 1999.
63. Lundgren C., Bergø G., and Tyssebotn I. Perfluorocarbon-stabilized intravascular microbubbles: an ultra-effective mode of oxygen delivery. Eighth International Symposium on Blood Substitutes, San Diego, California, November 9-11, 2000.
64. Tyssebotn I., Bergø G., and Lundgren C. Intravascular perfluorocarbon-stabilized microbubbles for treatment of hypoxemia due to an experimental intrapulmonary shunt. Eighth International Symposium on Blood Substitutes, San Diego, California, November 9-11, 2000.

65. Lundgren C., Bergø G. W. and Tyssebotn I. Tissue denitrogenation by oxygen breathing is enhanced by perfluorocarbon-stabilized intravascular microbubbles. Undersea and Hyperbaric Medical Society Annual Scientific Meeting, San Antonio, Texas, June 13-16, 2001.
66. Tyssebotn I., Bergø G.W. and Lundgren C. Volume-stabilized intra vascular microbubbles provide vital oxygenation in hemoglobin depleted pigs during "air breathing". Undersea and Hyperbaric Medical Society Annual Scientific Meeting, San Antonio, Texas, June 13-16, 2001.
67. Tyssebotn I., Bergø G. W. and Lundgren C. Extremely low doses of dodecafluoropentane emulsion sustain life and function in erythrocyte depleted animals. International Society on Oxygen Transport to Tissue 29th Annual Meeting, Philadelphia, PA, August 11-15, 2001.
68. Bergø G.W. and Tyssebotn I. Cardiac performance during hyperoxia. International Society on Oxygen Transport to Tissue 29th Annual Meeting, Philadelphia, PA, August 11-15, 2001.
69. Lundgren C., Bergø G. and Tyssebotn I. Extremely low doses of dodecafluoropentane emulsion sustain life and function in erythrocyte depleted animals. Advanced Technology Applications for Combat Casualty Care, Fort Walton Beach, FL, September 9-14, 2001, p. 16.
70. Hickey D.D., Tyssebotn I., Bergø G., Warkander D.E., Buyea C.M., Lundgren C.E.G. New method and device for measuring left atrial pressure: Theory and experimental verification. Advanced Technology Applications for Combat Casualty Care, Fort Walton Beach, FL, September 9-14, 2001, p. 16.
71. Hickey D.D., Tyssebotn I., Bergø G., Warkander D.E., Buyea C.M., Lundgren C.E.G. Minimally invasive method for measuring left atrial pressure. SCCM Critical Care Congress 2002, San Diego, CA, January 26-30, 2002.
72. Lundgren C., Bergø G. and Tyssebotn I. The theory and application of intravascular microbubbles as an ultra-effective means of transporting oxygen and other gases. IVth International Symposium on Current Issues in Blood Substitutes Research. Stockholm, Sweden, June 5-8, 2002. Abstract p.40.
73. Tyssebotn I., Bergø G.W. and Lundgren C. Intravascular microbubbles: successful treatment of potentially fatal hemorrhagic shock in pigs. IVth International Symposium on Current Issues in Blood Substitutes Research. Stockholm, Sweden, June 5-8, 2002. Abstract p.41.
74. Lundgren C., Bergø G. and Tyssebotn I. Oxygen delivery by intravascular microbubbles sustains life in a hemorrhagic shock model. Advanced Technology Applications for Combat Casualty Care, St. Pete Beach, Florida, September 9-13, 2002.
75. Lundgren C., Taraldoy T., Edstrom T., Tyssebotn I., Hickey DD. Mean left atrial pressure measured noninvasively in simulated microgravity. Abstract. 5th International Head-Out of Water Symposium: Research simulations to model microgravity. Houston, Texas, October 8-9, 2002.
76. Lundgren C., Bergø G. and Tyssebotn I. Hemorrhagic shock in air breathing pigs treated with bubble-forming intravenous dodecafluoropentane emulsion. Artificial Blood, 11: 1, abstract F-1-4, 2003.
77. Lundgren C., Bergø G. and Tyssebotn I. Intravascular microbubbles: an ultra-effective means of transporting oxygen. Keio J Medicine, 52: suppl. 1, p 16, 2003.
78. Lundgren CE, Bergø GW, Tyssebotn I. Long term Survival of Potentially Lethal Hemorrhagic Shock after Treatment with Intravascular Microbubbles. Abstract. Undersea Hyperbaric Medical Society 2004 Annual Meeting. May 24-28, 2004.
79. Tyssebotn I., Bergø GW, Lundgren CE. Treatment of Hemorrhagic Shock With Oxygen Carrying Microbubbles: Longterm Survival In Pigs. Abstract. American Heart Association Meeting. New Orleans, November 7-10, 2004.
80. Tyssebotn I., Bergø GW, Lundgren CEG. Delayed Treatment of Hemorrhagic Shock with Oxygen Carrying Microbubbles in Pigs. AHA-Resuscitation Science Symposium in Dallas, TX, Nov 2005, Circulation (Suppl) 111, 2005.

Popular Scientific Papers

1. Bergø, G. W. and Tyssebotn, I.: Blodstrømsfordelingen i kroppen og hjertet s arbeid ved varierende oksygeninnhold i pustega ssen. Vit enskaplig FUDT-seminar innen dykkemedisin/fysiologi, Bergen, Norway, pp. 20-23, 1990.
2. Stuhr, L.B., Bergø, G. W., Risberg, J. and Tyssebotn, I. Effekten av ulike pust egasser på hjertets pumpetrykk og kontraktilitet. Vitenskaplig FUCT-seminar innen dykkemedisin/fysiologi, Bergen, Norway, pp. 49-52, 1990.
3. Tyssebotn, I. Hvordan vil hyperoxi influere på utvaskning av inertgass og nødvendig dekompresjonstid? Vitenskapelig FUDT- seminar, Bergen, Norway, pp. 16-17, 1990.
4. Tyssebotn, I. and Ask, J.A. Det ytre trykks innvirkning på hjertemuskulaturen hos dyr og mennesker. Vitenskapelig FUDT-seminar, Bergen, Norway, pp. 45-48, 1990.
5. Tyssebotn, I. Sirkulasjonsforandringer hos dyr som utfører simulerte dykk. Populærvitenskapelig FUDT-seminar, Bergen, Norway, pp. 76-77, 1990.
6. Tyssebotn, I. Hvorfor trenger vi dykkfysiologisk forskning på dyr? Hva kan overføres til menneske fra slike forsøk? Populærvitenskapelig FUDT-seminar, Bergen, Norge, p. 70, 1990.
7. Hope, A., Bergø, G.W., and Tyssebotn, I.: Trykkfall og gassbobler i våre kne rotter. Vitenskaplig FUDT-seminar, Bergen, Norway, 1992.
8. Bergø, G.W., Hope, A., and Tyssebotn, I.: Evaluering av dekompresjon fra metning hos våre rotter. Vitenskaplig FUDT-seminar, Bergen, Norway, 1994.
9. Tyssebotn, I. De øvre luftveiers anatomi, fysiologi og spesifikke forsvarsmekanismer. Symposium om øvre luftveisinfeksjoner, Abbott, Oslo, 1990.